5

Environmental Effects of Ozone and Related Photochemical Oxidants

5.1 Introduction

Analyses of photochemical oxidants in the ambient air have revealed the presence of a number of phytotoxic compounds, including ozone (O_3), peroxyacyl nitrates (PANs), and nitrogen dioxide (NO_2). Ozone, the most prevalent photochemical oxidant, has been studied the most, and its effects are understood better than those of other photochemically derived oxidants. Ozone affects vegetation throughout the United States, impairing crops, native vegetation, and ecosystems more than any other air pollutant (Heck et al., 1980). The phytotoxicity of nitrogen oxides has been assessed in Air Quality Criteria for Oxides of Nitrogen (U.S. Environmental Protection Agency, 1993) and will not be discussed here. On the basis of concentration, the PANs are more toxic than O_3, with peroxyacetyl nitrate (PAN) being about 10 times more phytotoxic than O_3 (Darley et al., 1963; Taylor and MacLean, 1970; Pell, 1976). Although more phytotoxic than O_3, PANs generally occur at significantly lower ambient concentrations and are distributed less widely than those of O_3. Ambient concentrations of O_3 and PAN, as well as their concentration ratios, are discussed in detail in Chapter 4.

The effects of photochemical oxidants were observed first as foliar injury on vegetation growing in localized areas in Los Angeles County, CA (Middleton et al., 1950). In these early reports, foliar injury was described as glazing, silvering, and bronzing of the lower leaf surface of leafy vegetables and as transverse bands of injury on monocotyledonous species. Subsequent studies showed that these symptoms of photochemical oxidant injury were caused by PAN (Taylor et al., 1960). The characteristic O_3 stipple on grape (*Vitis labruscana*) leaves reported in the late 1950s was the first observation of O_3 injury to vegetation in the field (Richards et al., 1958). Subsequent studies with tobacco (*Nicotiana tabacum*) and other crops confirmed that O_3 was injuring vegetation at sites near urban centers (Heggestad and Middleton, 1959; Daines et al., 1960). It now is recognized that vegetation at rural sites may be injured by O_3 transported long distances from urban centers (Edinger et al., 1972; Heck et al., 1969; Heck and Heagle, 1970; Wolff et al., 1977a,b,c, 1980; Wolff and Lioy, 1980; Kelleher and Feder, 1978; Miller et al., 1972; Skelly et al., 1977; Skelly, 1980; Garner et al., 1989; see also Chapters 3 and 4). Concentrations of O_3 in polluted air masses often remain high for prolonged periods in rural areas, increasing the concern over possible effects on agriculture, forests, and native ecosystems.

Exposure to tropospheric O_3 can cause injury and premature mortality of plant tissues after entering the plant because O_3 has strong oxidizing properties and reacts with

cellular components. The effects of O_3 on terrestrial ecosystems begin with the responses of individual plants (Figure 5-1). Effects are initiated within the plant by reactions between O_3 or its metabolites and cellular constituents that influence biochemical and physiological processes and alter plant growth. Plant sensitivity to O_3 varies widely among individuals and among species. Sensitivity is determined both by genetic composition of the plant and environmental conditions. Plant response also is influenced by factors such as pollutant concentration, duration of exposures, plant nutrition, developmental stage, climate, insects, and diseases (See Sections 5.3 and 5.4).

Changes in foliar pigmentation and development of injured tissues are usually the first visible sign of injurious O_3 exposures and indicate impairment of physiological processes with the leaves. To affect metabolic processes within the cell, sufficient amounts of O_3 from the atmosphere must be able to enter the plant through the leaf stomata and dissolve in the aqueous layer lining the air spaces. Ozone and its decomposition products then diffuse through the cell membrane, where they can react with cellular components (unless the plant is able to detoxify or metabolize O_3 or its metabolites) (Section 5.3; Tingey and Taylor, 1982).

Ozone can affect all aspects of plant growth (Figure 5-1). Plants accumulate, store, and use carbon compounds to build their structure and maintain physiological processes (Waring and Schlesinger, 1985). Within the leaf, carbon dioxide (CO_2) absorbed from the atmosphere is converted to carbohydrates during the process of photosynthesis. The water and minerals necessary for growth are absorbed by plants from the soil. Growth and seed formation depend not only on the rate of photosynthesis and uptake of water and nutrients, but also on the subsequent metabolic processes and the allocation of the carbohydrates produced during photosynthesis. Most plants require a balance of resources (i.e, energy, water, mineral nutrients) to maintain optimal growth, but these are seldom available in natural environments (Chapin et al., 1987). Plants compensate for injury or stress by allocating their available resources to the point of injury or stress (McLaughlin et al., 1982; Miller et al., 1982; Tingey et al., 1976b). Altering the allocation of carbohydrates has been shown to decrease plant vigor, to increase susceptibility to insect pests and fungal pathogens, to interfere with mycorrhizal formation, and to reduce plant growth and reproduction (McLaughlin et al., 1982; Miller et al., 1982; U.S.Environmental Protection Agency, 1986; Garner et al., 1989).

Most of the available information concerning the effects of O_3 on vegetation is the result of exposure-response studies of important agricultural crops and some selected forest tree species, usually as seedlings. Through the years, crop plants, because of human food demand, usually have been selected for their productivity. They are grown as monocultures, fertilized, weeded, and frequently irrigated. In other words, competition for water nutrients, space, and light is minimized greatly when compared with plants growing in natural conditions, particularly in ecosystems. Trees for timber and paper also are grown on plantations under conditions favoring the greatest production.

Some O_3 exposures (concentration and duration) result in visible foliar injury to the plant without growth reduction; other exposures result in growth reduction and decrease in productivity without visible injury, whereas some exposures result in both. Data is presented in Section 5.6 that deals with the impact of different concentrations and exposure durations from many different experimental exposure-response studies on the growth of a variety of cultivated crops, ornamental species, and natural vegetation.

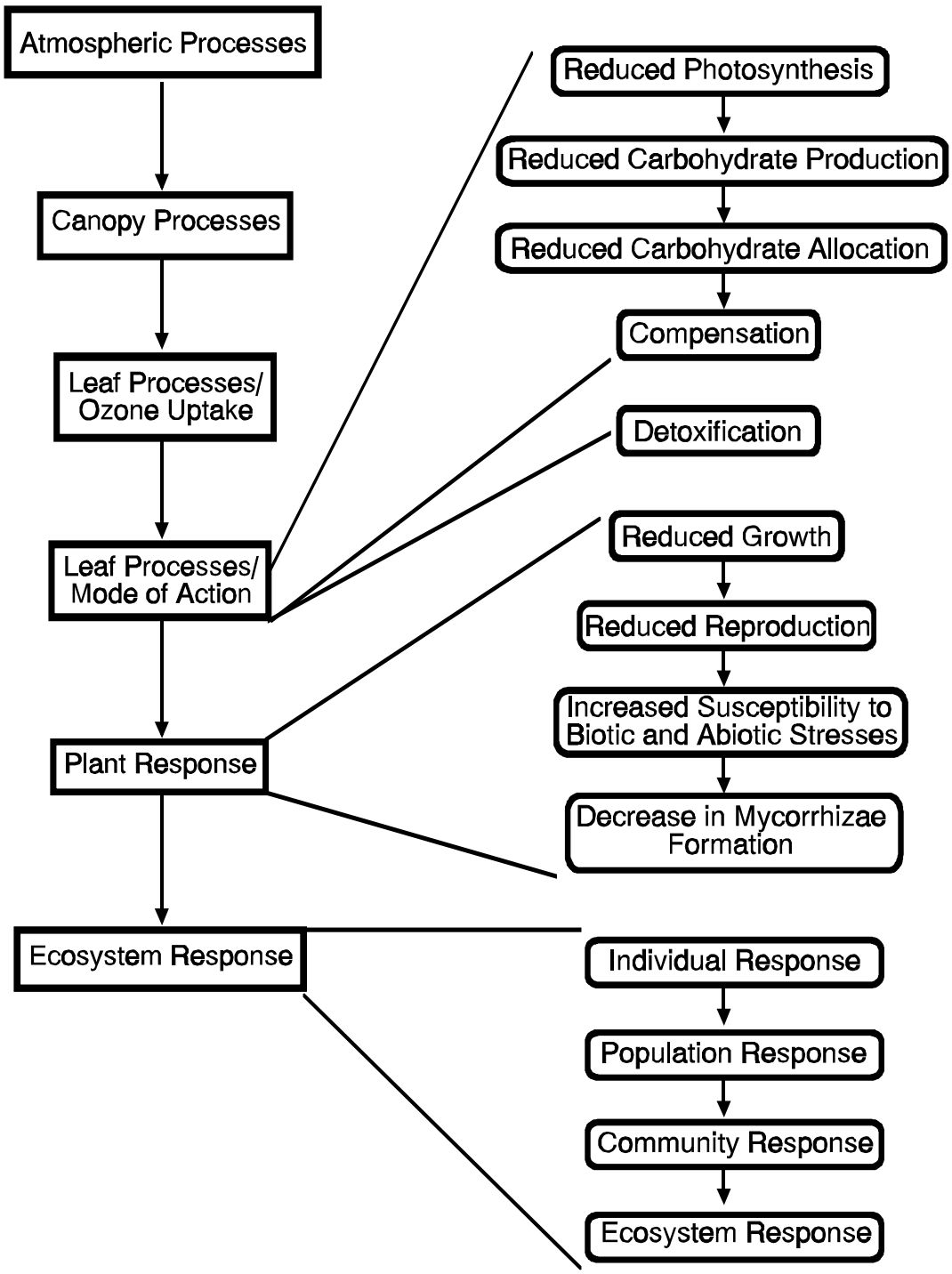

Figure 5-1. Leaf absorption and possible functional changes that may occur within the plant. Ecosystem response begins at the level of the individual and is propagated to the more complex level of organization.

The number of crop species and cultivars for which information regarding O_3 effects exists encompasses a mere fraction of the total of those cultivated as crops or found growing in natural communities. It is not possible to predict the sensitivity of the species and cultivars that have not been investigated, except in very general terms, because of the wide range of sensitivities to O_3 known to exist among crop cultivars and species that have been studied. Attempts to develop a general framework of response covering a range of species using the fragmented knowledge available have not been successful.

For many years, attempts have been made to develop mathematical equations that quantify the relationship between pollutant exposure and agricultural crop yield. The advantages and limitations of the various indices that have been developed to aid in predicting the effects of O_3 on crop yield are discussed in Section 5.5.

Organisms, not ecosystems, respond to O_3 exposure (Sigal and Suter, 1987). The only well-documented study of ecosystem change is that of the San Bernardino Mixed Forest ecosystem in Southern California where the impact of O_3 on the keystone species, ponderosa and Jeffrey pine (*Pinus jeffreyi*), resulted in the reversion of the forest to a simpler stage (Miller et al., 1982; Miller, 1984; U.S. Environmental Protection Agency, 1978, 1986). In other regions of the United States, most of the data available for assessing ecosystem responses deals with the responses of individuals to experimental O_3 exposures. Studies within the forests of the eastern United States, have dealt chiefly with the response in the field of eastern white pine (*Pinus strobus*) (McLaughlin et al., 1982; Skelly, 1980; Skelly et al., 1984). No long-term studies exist that deal with the impacts of O_3 on the various ecosystems components and how and whether these impacts alter ecosystem structure and functions. Therefore, the determination of the impact of O_3 on eastern forest ecosystems is difficult, if not impossible (see Section 5.7).

Plant populations are affected if they include many sensitive individuals. Removal of sensitive individuals within populations, or stands, if large in number, ultimately can change community and ecosystem structure (Figure 5-1). Structural changes that alter the ecosystem functions of energy flow and nutrient cycling can arrest or reverse ecosystem development (Odum, 1985).

The sequential organization of this chapter begins first with the methodologies (Section 5.2) that have been used to obtain the information presented and discussed in this chapter. Next, Section 5.3 explains the known biochemical and physiological changes that occur within the leaf cells after O_3 entry into the plants and how these chemical responses affect plant vigor, growth, and reproduction. Factors within and external to plants influence their response to O_3 and other stresses. These factors, as observed during experimental exposures and in the field, can modify functional growth responses of plants to O_3 (see Section 5.4). The development of indices or exposure statistics that may be used in quantifying and predicting crop responses to O_3 exposures are found in Section 5.5. Data obtained from many experimental exposure-response studies using methodologies presented in Section 5.2 and the basis for the development of the indices discussed in Section 5.5 are presented in Section 5.6. The information available on the ecosystem effects of O_3 and the data needed for more definitive assessments are found in Section 5.7. The costs to the nation of O_3 exposure of crops and ecosystems is discussed in Section 5.8. The scientific names of the plants cited in this chapter are presented in Appendix B. Section 5.10 discusses the effects of O_3 on nonbiological materials.

5.2 Methodologies Used in Vegetation Research

5.2.1 Fumigation Systems

The methodologies used in vegetation research have become more sophisticated over the years as new technology has developed. New exposure systems have been devised with pollutant dispensing systems that make it possible to more nearly duplicate the exposures plants receive in the field. These systems and their good points and shortcomings are discussed below.

Ozone fumigation plant-response studies require the fumigation of well-characterized vegetation to varying O_3 regimes. The variation in O_3 regimes may be achieved by controlled fumigation, chemical/mechanical exclusion or natural gradients of O_3. Controlled O_3 fumigation systems are designed to maintain a modified gaseous atmosphere around a plant for a period of exposure, for the purpose of monitoring plant responses to that modified gaseous atmosphere. All fumigation systems share some common features: general plant growth conditions (light, temperature, humidity, CO_2, and soil moisture) must be met, and differential concentrations of O_3 generated either artificially or naturally must be supplied to the vegetation and maintained during the exposure period. Exposure systems have been established in controlled environments, greenhouses, and the field. Many of these were described in the earlier criteria document, Air Quality Criteria for Ozone and Other Photochemical Oxidants (U.S. Environmental Protection Agency, 1986). More recent reviews of wet and dry deposition exposure systems have refined the knowledge of the strengths and limitations of experimental approaches for studying the effects of O_3, alone or in combination with other pollutants, on crops and trees (Hogsett et al., 1987a,b; Grünhage and Jäger, 1994a; Manning and Krupa, 1992). Controlled fumigation systems may range from cuvettes, which enclose leaves or branches (Bingham and Coyne, 1977; Legge et al., 1978), to a series of tubes with calibrated orifices spatially distributed over a field to emit gaseous pollutants to a plant canopy (Lee et al., 1978). Systems that exclude O_3 by mechanical or chemical means have been used, as have natural gradients of O_3, to evaluate vegetation response to ambient O_3.

5.2.1.1 Methodologies Discussed in the Air Quality Criteria for Ozone and Other Photochemical Oxidants (U.S. Environmental Protection Agency, 1986)

Controlled Environment Exposure Systems

Controlled environment fumigation systems are those in which light sources and control of temperature and relative humidity are artificial. Light quality and quantity are likely to be lower than in ambient environments, usually resulting in lower photosynthetically active radiation (PAR). Temperature and relative humidity likely will be more consistent in a controlled environment than in ambient air. Controlled environment exposure systems are typified by the widely used continuous stirred tank reactor (CSTR), a system originally designed for mass balance studies of O_3 flux to vegetation. The CSTR chambers have distinct advantages for gas exchange studies because fluxes can be calculated readily when controlling for environmental and pollutant conditions. The rapid air mixing minimizes horizontal and vertical gradients within chambers as well as leaf boundary layer resistance. Disadvantages of CSTR chambers include the following: the artificial pollution and growing conditions may not represent natural exposure conditions, the rapid air movement may cause wind injury to sensitive plants, the size of chambers restricts the study of large plants, and lighting systems are problematic and provide subambient levels of PAR. Although CSTR

chambers are useful for evaluating O_3 effects on physiological processes, it is not possible to extrapolate the data to field situations.

Greenhouse system designs are similar to those found in controlled environments, except that light, temperature, and relative humidity conditions fluctuate with those occurring in the greenhouse. Thus, greenhouse system designs are related more closely to field studies than are controlled environments, but plant culture and environmental conditions are still quite different from those of field exposure chambers, making direct extrapolation difficult. These studies are, however, more applicable to phytotoxicity of O_3 to greenhouse grown ornamental and floriculture crops (U.S. Environmental Protection Agency, 1986). Some greenhouse exposure systems use activated charcoal filtration to remove pollutants from the incoming air prior to the addition of experimental O_3 and either vent directly to the outside or use charcoal filtration of the outgoing air to prevent contamination of the greenhouse air supply. Other greenhouse exposure systems filter neither incoming nor outgoing air.

Field Exposure Systems

Fumigation of plants with O_3 in the field is most frequently carried out using open-top chambers (OTCs). There are many designs, each produces an environment that differs in some degree from the ambient air (Unsworth et al., 1984a,b). The most widely utilized design (U.S. Environmental Protection Agency, 1986) consists of a cylindrical aluminum frame, covered with transparent film. The bottom half of the transparent covering is double layered, with the inside panel perforated. Charcoal- and particulate-filtered air, nonfiltered air, or O_3-supplemented air is blown into the bottom layer, forced through the perforations into the plant canopy, and then escapes through the top of the chamber. The positive pressure maintained by the forced movement of air up through the chamber minimizes influx of ambient air into the chamber through the open top. The design of these chambers has been modified with frusta to reduce such incursions by ambient air, making the chambers more viable under windy conditions. Moveable canopies have been added so that rain exclusion studies can be carried out. Finally, these chambers have been modified in shape or increased in size so that species such as mature trees and grapevines can be enclosed. The OTC exposure system was employed in the National Crop Loss Assessment Network (NCLAN) from 1980 to 1988, and a description and discussion of the chambers is provided in Section 6.2.4 of the 1986 criteria document (U.S. Environmental Protection Agency, 1986).

The main advantage of OTCs is the ability to provide an enclosed environmental area for an increased range of treatments at near-ambient environmental conditions, while excluding ambient pollutants. Most current OTC designs have been used widely and successfully for studying the impact of O_3 on crops over a growing season (e.g., NCLAN program), but have diameters and heights that limit their use for larger plants. Although the OTCs provide for the least amount of environmental modification of any outdoor chamber, the OTC still may alter the microclimate sufficiently to have a significant effect on plant growth under pollutant stress. The OTC effects on the microclimate include reductions in light intensity, wind velocity, rainfall, and dew formation and persistence, and increases in air temperature and possibly relative humidity (Hogsett et al., 1987a; Heagle et al., 1988a; McLeod and Baker, 1988; Heck et al., 1994). For plants taller than 120 cm, there is more air movement near the bottom of the plant canopy than near the top during calm periods (Heagle et al., 1979c; Weinstock et al., 1982).

Exhaustive comparisons have been made among plants grown in carbon-filtered (CF) chambers, NF chambers, and similarly sized and located ambient air (AA) plots. Much attention has been paid to the potential for differences in productivity between AA and NF plants because of the modification of microclimate in OTCs (Manning and Krupa, 1992). For NCLAN studies, plants in NF chambers were frequently taller than AA plants (Albaugh et al., 1992; Olszyk et al., 1980; Heagle et al., 1979b). However, height was the only variable that was consistently different between AA and NF (Heagle et al., 1988a). Krupa et al. (1994) demonstrated that of 73 comparisons between NF and AA plants (NCLAN data), 56 showed no statistical significance, due either to lack of chamber effect or to random compensation. A more relevant question, whether OTCs change plant response to O_3, has been addressed. A comparison of plant growth and plant response to O_3 exposure in OTCs, closed-top chambers, and air-exclusion systems has been carried out (Olszyk et al., 1986a). The authors discovered that there was interaction between plant response to O_3 and type of exposure system for less than 10% of the growth parameters measured in California, suggesting that plant response to O_3 was the same regardless of exposure system. Plants from exclusion systems were shorter than those grown in OTCs and generally weighed more. Of the three groups of plants, those in the control plots of the exclusion system (i.e., receiving ambient O_3exposure) were most similar in size to plants grown in field plots. Although this and another study (Olszyk et al., 1992) indicate that environmental modification caused by chambers will affect plant growth and yield, there is no evidence that there is a large effect of chambers on plant response to O_3. It is assumed that, because of the decreasing relative effects on plant environment caused by controlled environment, greenhouse, closed-top field chambers, OTCs, open-air systems, and ambient gradients, the system effects on plant response to O_3 will decrease in the same order. Microclimatic differences within an OTC can cause significant differences in yield, but rarely were there significant interactions between position effect and plant response to O_3 (Heagle et al., 1989a).

Considerable concern has been raised about plant response to trace pollutants in OTCs, specifically nitrogen pentoxide (N_2O_5) and nitric oxide (NO) in chambers receiving O_3 generated from dry air, and NO_2 in chambers receiving AA. These trace pollutants may have a direct effect (positive or negative) on plant processes or may change how plants respond to O_3, and, without careful evaluation, these effects may go undistinguished from those of O_3. A comparison of alfalfa (*Medicago sativa*) response to the same O_3 exposure, generated either electrostatically from air or through nonfiltration of AA, indicated that the generated O_3 treatment was more phytotoxic than the ambient O_3 treatment, probably due to the co-generation of N_2O_5 and NO, along with O_3 from dry air (Olszyk et al., 1990a). Open-top chamber studies that use filtered versus NF ambient O_3 have been proposed to avoid the problems of generating O_3. The drawback of this or any two treatment approaches is that such plant responses to low ambient levels of O_3, such as might occur in many years, is quite subtle. To detect statistically significant differences between filtered- and NF-chamber-grown plants when responses are subtle requires a high number of replications (Rawlings et al., 1988a). This fact is illustrated in Heagle's own two-chamber work; as described in Heagle (1989), some of the two-chamber studies had differences between AA and NF of greater than 10%. Such large differences reduce the number of replications needed to detect a significant difference at $p = 0.05$. In any event, the differences either were not treated nor tested, or were tested but were not significant, except in one case at Beltsville, MD, with soybean (*Glycine max*). Heagle (1989) discussed the calculation of power and reviewed two-chamber studies in great detail.

Limited use (for O_3 studies) has been made of chamberless field exposure systems, which rely on ambient wind conditions to move O_3 across an open-field canopy. The O_3 is emitted from vertical pipes, which are spaced in a circle around the experimental plot of plants. The amount of O_3 emitted from each vertical pipe, as well as the number and compass direction of emitting pipes, depends on the wind direction and speed; this whole process is usually being computer controlled.

5.2.1.2 Methodologies Referenced Since the Air Quality Criteria for Ozone and Other Photochemical Oxidants (U.S. Environmental Protection Agency, 1986)

Branch and Leaf Chambers

Most of the developments in exposure systems since 1986 have been modifications of existing systems. The tremendous interest in evaluation of mature tree response to O_3 has prompted the development of large branch chambers for estimating O_3 flux to trees. These branch chambers share many of the design characteristics of a CSTR. The chamber walls are transparent film spread over a supporting frame. There is a fan to reduce boundary layer resistance across the foliar surface, and an air inlet and outlet so that differential O_3, CO_2 (photosynthesis), and water vapor (leaf diffusive resistance) measurements can be taken (Ennis et al., 1990; Houpis et al., 1991; Teskey et al., 1991). The advantages of this system include the ease with which the Teflon® bag can be replaced; uniform light transmission can be maintained; and the branch chamber can be moved from plant-to-plant, can be used in situ, and can be modified for different sized branches. One of the disadvantages of the branch chamber, and indeed of any such cuvette that isolates one part of the plant under different environmental conditions than the rest of the plant, is that the isolation may lead to a response different from that which would have been observed if the branch was under the same environmental conditions as the rest of the plant. In addition, total tree growth cannot be estimated using branch chambers because only part of the plant is treated with O_3.

Flux Measurement

Estimation of O_3 flux to foliage can be made directly by measuring the difference in O_3 concentration between air going into a leaf chamber and the same air stream exiting the chamber after passing over the leaf. This estimation also can be inferred from measurements of leaf diffusive resistance during exposure of a leaf to O_3. The former method requires a chamber or cuvette fumigation system with uptake of O_3 that is quite small or extremely nonvariable relative to the amount being taken up by the leaf. Otherwise, it is difficult to detect O_3 flux to a leaf with good precision. Such cuvettes can be adapted from those commercially available for portable photosynthesis meters (Graham and Ormrod, 1989) or constructed from a novel design, such as that developed by Fuentes and Gillespie (1992) to estimate the effect of leaf surface wetness on O_3 uptake of maple leaves. The criteria for flux cuvette design include good light transmissibility, ease of leaf manipulation, minimal reaction of chamber wall surface with O_3, and good air mixing within the chamber. Good mixing of air is necessary to avoid a gradient in pollutant concentration and to maintain a boundary layer resistance, which is much less than stomatal resistance. Maintenance of leaf temperature close to that of the surrounding air, so that transpiration rates are not abnormally high, is another benefit of good air mixing. The physical design of the Fuentes and Gillespie chamber was simple, consisting of two glass hemispheres that were clamped together and separated by a Teflon® O-ring over the petiole of the leaf under investigation. Inlet and outlet

air attachments were on opposite sides of the cuvette. Other cuvette designs have been used to estimate leaf gas-exchange responses to O_3; their principals of operation are similar, but there are differences in materials and design (Amiro et al., 1984; Freer-Smith and Dobson, 1989; Laisk et al., 1989; Moldau et al., 1991a; Skarby et al., 1987).

Compared to the CSTR, which has been used for mass balance measurement of gas flux by whole plants during fumigation (Le Sueur-Brymer and Ormrod, 1984), cuvette systems usually determine flux to one leaf at a time. This results in a more precise understanding of the interaction among leaf age, diffusive resistance, illumination and O_3 flux. However, these data are not particularly well adapted to estimating flux of O_3 to a large vegetated surface. Finally, regardless of the methodology used to determine O_3 flux to foliage, there exist only very sketchy mechanistic-process models that would link O_3 fluxes to decreases in growth and productivity of plants. These data primarily are useful for developing a relationship between *internal* O_3 dose and plant response and in estimating the strength of vegetation as sinks for O_3 flux on a large scale. Recent studies have estimated fluxes of O_3 to plant canopies by indirect methods. Ozone flux to oat (*Avena sativa*) in OTCs (using mass balance principles and a resistance analogue model) was compared to that for oat growing in the field, using an aerodynamic gradient method (Pleijel et al., 1994). Vertical flux density calculations for O_3 uptake by grassland vegetation (O_3 based on radiometric measurements) estimated exchange between the atmosphere close to the ground and the ecosystem (Grünhage et al., 1994; Dämmgen et al., 1994). Although fluxes of O_3 to vegetation cannot imply growth or O_3 physiological responses, techniques such as these can suggest whether plant responses to O_3 in OTCs might differ from those in ambient field culture because of micrometeorological-induced differences in O_3 flux.

Pollutant-Dispensing Systems

Although exposure chambers have changed little in design in the last several years, the profile characteristics and method of dispensing pollutant profiles have. Whereas early studies utilized static or square-wave exposures, usually controlled by hand-set flowmeters, many more recent systems expose plants with so-called dynamic exposures during which the O_3 concentration gradually reaches a maximum, thus simulating diurnal variation in O_3 concentration (Hogsett et al., 1985a). These profiles may be achieved by mass flow controllers that are themselves computer controlled. Proportional-add systems such as that used in NCLAN usually achieve ambient type profiles using rotameters instead of mass flow controllers. The O_3 concentration in each of the chambers is logged at preset intervals, so that the integrated exposure for the entire fumigation period can be calculated. Deviations from the planned O_3 episode can occur, due to failure in dispensing or monitoring equipment, as well as incursions of air through the tops of the chambers. The length of the interval between determinations of O_3 concentration in the chambers can be an important contribution to the control of O_3 profile. In general, longer intervals lead to less well-controlled and well-characterized O_3 exposure profiles (Lefohn et al., 1993). These deviations from the expected profiles can be mathematically quantified and monitored among treatments and replications (Hale-Marie et al., 1991).

Open-Air Field-Fumigation Systems

Open-air field-fumigation systems have the potential to estimate most closely field losses due to O_3, as the plants are grown and exposed under ambient field environmental conditions. However, of all the fumigation systems, this is the least controllable and

repeatable. It has been used in the past to expose plants to "static" concentrations (i.e., desired concentration is the same throughout the exposure period) of such pollutants as sulphur dioxide (SO_2) or hydrogen fluoride (HF) (Hogsett et al., 1987a). The Zonal Air Pollution System (ZAPS) has been modified vastly and improved on to enable fumigation of plants with a diurnally varying pattern of concentration (Runeckles et al., 1990). The system represents a significant advancement over earlier open-air field fumigation systems in that 12 discrete seasonal treatments that simulate ambient patterns are achieved, rather than the usual two or three. Ozone was supplied to 4-m plots, which were laid out in groups of four, through a manifold suspended over the plant canopy. The wind speed and direction determined the actual seasonal O_3 exposures, although the O_3 was released in concentrations proportional to that observed at the time in the ambient environment. Although the 12 treatments are not repeatable over time, a regression relationship between pollutant exposure and plant response can be established for each growing season.

The Liphook study in England of long-term responses of *Picea sitchensis*, *Picea abies*, and *Pinus sylvestris* to SO_2 and O_3 in combination consisted of seven growth plots, 50 m in diameter, five of which were surrounded by 64 vertical pipes from which pollutant gasses were emitted (McLeod et al., 1992). The 64 pipes were divided into four quadrants of 16 adjacent pipes, and each quadrant had diluted pollutant gases supplied to it from a computer controlled mass flow controller. The emitting quadrants, as well as the rate at which the gases were supplied to the quadrants, depended on wind speed and direction. The gases were emitted from the vertical pipes into the plant canopy at two heights, 0.5 and 2.5 m above a reference height, which was approximately two-thirds of tree height. This pattern of gas dispersion resulted in a uniform horizontal distribution of hourly mean gas concentration across each central 25-m diameter experimental plot. This exposure system, like all open-air exposure systems, clearly simulates field plant growth conditions far better than open- or closed-top chambers, and, with five enclosures and two nonenclosed ambient plots, this is by far the largest of the very few of these systems that are in operation. Measured over a winter wheat canopy, SO_2 concentration differed by less than 1 $nL.L^{-1}$ over a 5-h period of measurement; measurement of consecutive 2-min mean values at five locations across the plots demonstrated high uniformity (McLeod et al., 1985). The usefulness of the data is limited, however, by the low number of treatments and lack of replication of those treatments.

Field Chamber Exposure Systems

Open-top field chambers are used in most field studies of plant response to gaseous pollutants. The OTCs first were designed for studies on annual herbaceous crop plants (Mandl et al., 1973), but enlarged versions also have been used successfully in tree seedling and sapling studies (Adams et al., 1990a,b; Chappelka et al., 1990; Qiu et al., 1992; Kress et al., 1992; Hogsett et al., 1989; Andersen et al., 1991; Karnosky et al., 1992a,b; Wang et al., 1986a,b; Temple et al., 1992). Because the results from these studies using tree species are extrapolated to predict the effects of O_3 on forests, these studies require good exposure control in order to replicate ambient O_3 profiles characteristic of many low-elevation, rural areas of eastern North America. This condition could have been met using an open-field exposure system. Open-top chambers large enough for mature trees have been developed but are expensive (Mandl et al., 1989; Albaugh et al., 1992).

Microclimatic modification by OTCs, as well as O_3 exposure schedules that are disconnected from typical O_3 episode meteorology, have been addressed in a seasonal study

of tree response to O_3 in the United Kingdom (Wiltshire et al., 1992). This study uses OTCs with roll-up sides, but, except for fumigation days, the plants are maintained in ambient climatic conditions. The exposure episodes number between 27 and 30 throughout the growing season and occur on days with ambient meteorology associated with naturally occurring O_3 episodes (i.e., high incident radiation and temperature, with little air movement) (Wiltshire et al., 1992). The maintenance of near-ambient meteorological conditions during both growth and exposure periods is an effort to make this study better represent field-grown plant responses to O_3, while maintaining control of O_3 exposure.

Several designs of field fumigation chambers have been developed to overcome some of the disadvantages of the OTCs, namely small plot size and incursion of ambient air. Closed-top chambers first were developed in the 1950s; generally, their use diminished in favor of OTCs. However, closed-top chambers smaller in dimension than the open-top design have been constructed more recently in California to assess crop loss to O_3. Closed-top chambers were chosen because the authors wished to characterize the pollutant dose to the plants very precisely; pollutant gradients within the chamber were minimal (Musselman et al., 1986a). The chambers were octagonal in shape and covered with Teflon® film; the soil was completely replaced with standard greenhouse mix. Temperatures in the chamber were higher (2 to 4 °C at midday, 1 to 2 °C at night) than in the ambient air, and light levels were reduced by 11% (spectral quality of the light in the chambers was not reported). The authors concluded that, although the chambers were not suitable for studies destined for extrapolation to plant response under field conditions, the chambers were very useful when tight control over soil moisture and pollutant concentration was needed.

Closed-top chambers were constructed and installed in the United Kingdom to study responses of shrubs and large herbaceous species to long-term, low (chronic) concentrations of SO_2, NO_2, and O_3 (Rafarel and Ashenden, 1991). These chambers were a smaller version of an earlier design, because the larger chambers required pure gas sources of NO_2 and SO_2 to be diluted into the ventilating air stream, which resulted in highly variable exposure concentrations. The flow rate of the smaller chambers meant that premixed gases were sufficient to maintain steady control of treatment concentrations. Because the gases were discharged from the source at constant concentrations, different treatments were achieved by placing one or more pollutant supply tubes in the fumigation chambers. Good air circulation and moderate ambient temperatures maintained the chambers at near ambient conditions; however, results cannot be extrapolated to predict plant response to O_3 under ambient air conditions.

Ambient Gradients for Evaluation of Plant Response to Ozone

The exposure system that utilizes ambient conditions of O_3 exposure, temperature, humidity, soils, and soil moisture is the ambient gradient system. By this method, plants are grown along a transect of known differential pollutant concentrations, usually downwind of a major point source or urban area. The concentration of pollutants is diluted as distance from the source increases. The most well-defined O_3 gradients exist in the Southern California Air Basin and have been used in studies by Oshima et al. (1976, 1977a,b); unfortunately, outside this region, few suitable gradients exist. A study using four different cultivars of red clover (*Trifolium praetense*) and spring barley (*Hordeum vulgare*), each differing in sensitivity to SO_2, NO_2, and O_3, was conducted along such a transect of gradient SO_2, NO_2, and O_3 concentrations in the United Kingdom (Ashmore et al., 1988). Ozone concentration was inferred from injury to Bel W3 and Bel B cultivars of tobacco but was found to have very

little relationship to cultivar performance. The authors cautioned that these results must be interpreted with an understanding that differences among sites in other environmental parameters could contribute to the detection of (or the failure to detect) O_3 effects on the crops. For ambient gradient studies to be interpretable, good characterization of site parameters (rainfall, temperature, radiation, and soil type) is needed. Additionally, the modeler needs to know how these factors should be used to adjust the apparent plant response. In order to know that, a good knowledge base is needed of how all of these factors modify plant response to O_3.

Although Manning and Krupa (1992) assert that natural gradients are the "ideal way to conduct O_3/plant response studies in ambient air in field plots," they concede that few gradients that meet statistical requirements for intermediate O_3 concentrations exist outside Southern California. It is possible, however, that more gradients will be identified as rural air monitoring increases. They also concede that, although using artificial soils removes a significant source of variation in plant response to O_3, pot-grown plants do not closely simulate the rooting environment found in the field (Manning and Krupa, 1992). Although plants using gradients are commonly considered to be easily replicable in large numbers, they should probably be considered as "repeats" rather than "replicates" in the conventional sense. If treatments are replicated by locating them very close together at the same location in the gradient, then they may better be considered as "sub-samples" of one replicate, if the climatic and edaphic conditions are very similar, or as repeats of a study, if the conditions are not. This argument is not just semantic; in data analysis, repeats and replicates should be handled differently, because the sum of squares for repeats is likely much larger than for replicates and may be composed significantly of plant response factors other than O_3 concentration.

At this time, although some information is available, the relationships still are incompletely understood. Many investigators consider that ambient gradients are impossible to find without major differences in environmental conditions that may affect plant response to O_3 and, therefore, confound interpretation of the results.

Cultivar Comparisons

The comparison of isogenic lines of a particular species that differ only in their tolerance to O_3 is "the ideal way to determine the effect of ambient O_3 on plants in the field ...." (Manning and Krupa, 1992). Heagle et al. (1994) report on the use of a white clover (*Trifolium repens* L.) system to estimate the effects of O_3 on plants. A field experiment conducted in 1984 and 1985 using white clover revealed a wide range of sensitivity among the genotypes present in the commercial line "Regal" (Heagle et al., 1991a, 1993). Plants were screened for relative sensitivity to O_3. Two clones were selected: one ozone-sensitive (NC-S) and another ozone-resistant (NC-R). Subsequent studies suggested that these clones could be useful as indicators of O_3 sensitivity, if they routinely displayed measurable differences in response to O_3, while responding similarly to other factors (e.g., biotic, climatic, soil, chemical, and other pollutants). Experimentation indicated that the white clover system can be used to indicate where and when ambient O_3 concentrations cause foliar injury and decrease growth. Hence, it can be inferred that other plant species sensitive to O_3 also may be affected (Heagle et al., 1994).

Protective Chemicals

Chemicals that protect plants from O_3 have been in use since the 1970s to evaluate plant response to O_3. Ethylene diurea (EDU) has been used in studies to modify the

O_3 sensitivity of several species (see Section 5.4.7). Ethylene diurea (and perhaps other undetermined chemicals) has potential as a tool to evaluate field crop losses to O_3 in the absence of chambers, with their inherent modification of microclimate. A low-cost, simple technique, EDU can be applied to larger plot sizes than currently are possible with OTCs, thus reducing some of the uncertainty of extrapolating experimental results to a large scale. Field protocols for the use of EDU have not been well established. Frequency and rate of application that protects plants vary with species and edaphic and atmospheric conditions. Depending on the method of application, EDU may have little effect on field-grown plant response to O_3 (Kostka-Rick and Manning, 1993). The basis for the year-to-year variation in degree of protection of plants by EDU is not well understood, so drawing conclusions from multi-year studies, which is the situation most relevant to evaluation of plant community responses to ambient O_3, is difficult. Two-treatment studies of EDU and plant response to O_3 (Kostka-Rick and Manning, 1992a,b) indicate that protection is variable, suggesting that the experimental system under investigation (soil, plant, and climate) would have to be extremely well characterized and understood for interpretation of EDU studies to be complete. Manning and Krupa (1992) point out that EDU is probably more useful in conjunction with OTCs so that a factorial range of O_3 can be administered to the plants. It is not clear that EDU protection can be fine-tuned sufficiently into a range of discrete levels suitable for regression analysis (Kostka-Rick and Manning, 1993). The mechanism by which EDU protects plants, beyond being a systemic antioxidant, is unknown; understanding this mechanism has the potential to contribute to the broader understanding of the mechanisms of O_3 injury at the cellular/metabolic level of the plant.

5.2.2 Experimental Design and Data Analysis

Experimental design strategies, including the number, kind, and levels of pollution exposure; patterns of randomization; number of replicates; and experimental protocol are crucial to the ability of the statistical approaches to test and model the effects of O_3 on plant response and to extrapolate experimental results to real world conditions. The experimental design focuses an experiment on the specific objectives of the study and, so, may limit the application of the data to other research goals. The various experimental design and analyses for exposure-response data from controlled exposure studies have been well reviewed in the 1986 criteria document (U.S. Environmental Protection Agency, 1986) and will not be repeated here. In summary, most field studies involving OTCs have used randomized block or split-plot designs and pollution levels appropriate for regression analysis. These exposure-response relationships generalize the mathematical relationship between the plant parameter of interest and O_3 exposure. Plant response to concentrations other than those used in the experiment can be interpolated from these relationships, and thresholds of plant response can be determined (Ormrod et al., 1988). In the latter half of the NCLAN program, the Weibull model was chosen to characterize yield response to O_3 because of its flexibility to describe a wide range of data patterns (Rawlings and Cure, 1985) and, consequently, to allow a common model to be fit when pooling data across years and sites (Lesser et al., 1990).

Experimental designs for exposure-response relationships can be expanded easily so that plant response to O_3 and another factor at multiple levels can be determined. Because of the need to contain each O_3 treatment by a chamber, incomplete factorial designs are more efficient approaches to multi-factor studies, leading to exposure-response surfaces (Allen et al., 1987). Choosing the appropriate incomplete factorial design for a response surface

study requires forethought on whether all areas of the surface are of equal interest. For many O_3 plant response studies, this is not so because extremely high concentrations, although increasing the precision with which plant response to lower concentrations is estimated, are not as likely to occur in the ambient environment (see Chapter 4).

Because the U.S. Environmental Protection Agency (1986) decided to place greater emphasis on damage (i.e., effects that reduce the intended human use of the plant) than on injury, studies more frequently have used experimental designs that generate data suitable for regression and treatment mean separation analyses for the purposes of modeling and testing the impact of O_3 on plant response. Although the impact at current O_3 levels is of primary interest and can be studied effectively using two O_3 levels generated by CF and NF treatments, the development of exposure-response models necessitates the use of additional treatments at above ambient concentrations (Heagle et al., 1989a; Rawlings et al., 1988a). The optimal number, range, and spacing of treatment levels depends on the anticipated exposure-response model, but, in the case of the Weibull and polynomial models, greater precision for estimation of relative yield loss at ambient O_3 concentration is obtained when the lowest treatment level is near zero and the highest treatment level is well above the ambient concentration. For the Weibull model, the highest treatment should correspond to a concentration for which yield loss is at least 63% of the yield at zero exposure (Dassel and Rawlings, 1988; Rawlings et al., 1988b).

When studying the impact of mixtures of pollutants on plant processes in chambers, response surfaces can be generated from complete or incomplete factorial designs. These designs have been shown to increase the precision and efficiency of estimating relative yield loss at ambient concentrations (Allen et al., 1987). The optimal design cannot be specified a priori and necessitates the use of treatment levels from near zero to well above the ambient concentration for each pollutant. However, response surface designs have not been used widely in pollutant mixture studies, nor have these designs been used extensively to study the interaction between pollutant exposure and quantitative environmental parameters, such as light, temperature, and soil moisture. The interaction between O_3 and phytotoxic concentrations of other pollutants, in particular SO_2, has not been studied extensively because instances of co-occurrence of O_3 and other pollutants are not common in the United States. An analysis of pollution monitoring data showed fewer than 10 periods of co-occurrence between O_3 and phytotoxic concentrations of SO_2 during the growing season at the sites where the two pollutants were monitored (Lefohn and Tingey, 1984; U.S. Environmental Protection Agency, 1986).

Design and analysis of pollutant effects studies have used various characterizations of exposure to determine optimum spacings of treatment levels and to relate exposure to response. Most notably, the daytime mean concentration index (i.e., either M7 or M12) was adopted by the NCLAN program to determine the effects of O_3 on plant response. However, there has been considerable debate over the use of the mean index in exposure-response modeling; the variety of ways to compute the characterizations of plant exposure will be discussed in Section 5.5. When plant yield is considered, plant response is affected by the concentration of exposure and by other exposure-dynamic factors (e.g., duration, frequency, threshold, respite time), in combination with physiological, biochemical, and environmental factors that may mask treatment effects over the growing period. Research goals to understand the importance of exposure dynamic factors have utilized experimental designs that apply two or more different patterns of exposure that are equal on some scaling (e.g., total exposure). Experiments designed specifically to address the importance of components

of exposure may require the use of exposure regimes that are not typical of the ambient environment.

The majority of chambered field studies use regression-based designs that focus on developing exposure-response models but have limited application for testing the importance of exposure dynamics (e.g., exposure duration) for evaluating exposure indices based on statistical fit. When data from replicate studies of equal or varying duration are available, the ability to test for duration effects on plant response may be enhanced using regression analysis to combine data. The regression approach has been used to fit a common model to combined data from replicate studies of the same species when it is reasonable to assume that the primary cause of biological response is pollutant exposure, and that differences in environmental, edaphic, or agronomic conditions among sites do not significantly change the shape of the regression relationships. When pooling data across sites and years, additional terms for site and year effects often are included in the model as either fixed or random components, depending on the population of interest. Inferences over random environments implies that the environments sampled by the experiments are representative of the population of regions of interest under a variety of environmental conditions. In this case, site and year effects are incorporated as random components when fitting a common model. The appropriate analysis is to use a mixed model to fit an exposure response model with variance components. This analysis has been used recently to combine data from replicate studies of varying durations to test the importance of length of exposure in influencing plant response (see Section 5.5).

5.2.3 Mechanistic Process Models

In addition to regression type models of plant response to O_3, which are empirical and statistical in nature, there are mechanistic-process models (Luxmoore, 1988; Kickert and Krupa, 1991; Weinstein et al., 1991). The key difference between these two types of models is how the changes are handled in the dependent variable over time. Empirical models treat a time period (e.g., a growing season) as a single point and report the response of the dependent variable as a single point as well. Regression models also may oversimplify the characteristics of an O_3 exposure, in that the description of the O_3 exposure is compressed over time to a single number. The variety of ways to compute this single number will be discussed in Section 5.5.

Mechanistic-process models on the other hand describe the rate of change of a variable in response to the treatment (such as O_3) with change in time. The latter type of model has the potential to capture the interaction among plant age or stage of development, variability of ambient exposure concentrations, and plant response to O_3. For this reason, mechanistic-process models have been rated much more highly than regression models for their realism, scientific value, and applicability to other locations (Kickert and Krupa, 1991). However, compared to regression models, mechanistic-process models require more input data, and the input data are less accessible. The mechanistic-process models are more complex than regression models, requiring more computer time and memory to develop. The precision of the output regression models is greater than mechanistic-process models (for interpolative examinations only), as is their ability to estimate response probabilities. The authors conclude that the popularity of single-equation, time-lumped models is related to the fact that the studies of plant responses to O_3 are oriented more to air quality standard setting as an endpoint, rather than the physiological processes underlying plant responses. The

problems with process-based models are the necessity for some large assumptions (in place of real data) and the lack of validation. Without validation, using estimates from these models is questionable; if the estimations are used, then the uncertainties associated with them must be identified and quantified.

5.2.4 Summary

Each type of fumigation system is suited particularly well to certain types of studies of plant response to O_3; no one system is appropriate for all types of studies of plant responses to O_3 (Table 5-1). Each system has advantages and limitations that must be evaluated in terms of the research objectives that it was designed to meet. Table 5-1 lists the characteristics of the various exposure systems as they relate to experimental objectives, including simulation of field conditions, replication, range of treatment levels possible, and the ability to control extraneous environmental factors that may influence plant growth. Controlled-environment chambers are well suited for mechanistic type studies at the molecular or cellular level. Most plant cellular processes, as well as the equipment that measures them, are quite sensitive to temperature and light, so good control and definition of these factors are needed. Growth responses to O_3 determined from controlled-environment chamber studies cannot be extrapolated to the prediction of field losses to O_3 because the culture conditions in the two systems are just too dissimilar. Open-top chamber systems, although a compromise in ability to simulate field conditions, have major advantages over other fumigation systems for developing exposure-response functions (to develop a statistically robust surface requires at least three or, better yet, five treatment levels) because (1) a range of pollution levels at near-ambient environmental conditions can be generated to optimize the precision in empirical modeling; (2) extrapolation of experimental results to probable field responses to ambient exposure is possible to a certain extent because OTCs, although modifying microclimate, appear not to affect relative plant response to O_3; and (3) a semi-controlled environment is created for plant growth with only O_3 exposure level varied, thus it is valid to assume that the primary cause of response is due to O_3 exposure. Exclusion methods, particularly those using chemicals such as EDU, are the least disruptive of ambient culture conditions in the field, so these approaches most closely estimate real crop losses to O_3. However, their application is limited by the availability of ambient O_3 in any particular year or location, as well as by confounding by climatic and edaphic conditions. They are not well suited for establishing exposure-response relationships because it is difficult to quantify the degree of protection actually offered by the exclusion method in the field (Ashmore and Bell, 1994). In general, open-field exposure systems or natural gradients are not replicable, nor can a range of treatments be imposed to enable construction of a response function, which is necessary for interpolation of O_3 concentrations that cause plant response.

At the current time, OTCs represent the best technology for determination of crop yield responses to O_3; concentration and duration of the gas are well controlled, and the plants are grown under near-field-culture conditions. There are several limitations and uncertainties associated with the collected data: (1) the plot size is small relative to a field, (2) microclimate differences may influence plant sensitivity to O_3, and (3) air quality after

Table 5-1. Comparison of Fumigation Systems for Ozone Exposure-Plant Response Studies

Fumigation System	Simulation of Field Losses	Replication of Experimental Unit	Range of Treatment Levels	Likelihood of Extraneous Factors Affecting Response
Controlled-environment chambers	Low	Low	High	Medium
Greenhouse chambers	Low	Medium	High	Medium
Closed-top field chambers	Medium	High	High	Medium
Open-top field chambers	Medium to high	High	High	Medium
Mechanical field exclusion	High	Low to medium	Low	Medium
Open-field fumigation	High	Low	Low	High
Natural gradients	High	Low	Low	High

passage through a charcoal filter has not been widely characterized. These uncertainties are not quantified, although there are preliminary data establishing their existence. There is concern that these uncertainties are forgotten in the scaling of the plant response data to national yields and their integration into larger cost-benefit models. However, because the uncertainties are not yet quantified, they cannot be incorporated into the national estimates of losses to O_3. There is an urgent need to estimate these uncertainties so that the OTC data can be used fully, with little doubt as to how well the data represents real crop losses. Further comparisons of OTC and chemical exclusion plant responses, expanding the range of environmental conditions and species for which they are compared, would help determine the extent of the role of microclimate in modifying plant response to O_3. Large scale exclusion studies also could contribute to quantifying the uncertainty of extrapolating plot response to field scale. Analysis of the atmospheric chemistry inside OTCs under various scenarios of light, temperature, and humidity would address the question of what additional pollutants may influence plant growth or plant responses to O_3. Once these uncertainties are fully characterized and quantified, existing models of crop loss can be constructed more precisely and then incorporated into the national scale models with greater confidence.

5.3 Species Response/Mode of Action

5.3.1 Introduction

Plant adaptation to changing environmental factors or to stresses involves both short-term physiological responses and long-term physiological, structural, and morphological modifications. These changes help plants to minimize stress and to maximize the use of internal and external resources. A great deal of information is available on the physiology of single leaves; however, relatively little is known about whole-plant systems and whether the physiological mechanisms involved are initiated wholly within the leaf or are the result of whole-plant interactions (Dickson and Isebrands, 1991).

The many regulatory systems contained in leaves change both as a function of leaf development and in response to different environmental stresses. Leaves function as the major regulators of anatomical and morphological development of the shoot and control the allocation of carbohydrates to the whole plant (Dickson and Isebrands, 1991). This section discusses the movement of O_3 into plant leaves and what is known about their biochemical and physiological responses.

Movement of O_3 into plant leaves involves both a gas and a liquid phase. The phytotoxic effects of air pollution on plants appear only when sufficient concentrations of the gas diffuse into the leaf interior and pass into the liquid phase within the cells. Therefore, to modify or degrade cellular function, O_3 must diffuse in the gas-phase from the atmosphere surrounding the leaves through the stomata into the air spaces and enter into the cells after becoming dissolved in the water coating the cell walls (U.S. Environmental Protection Agency, 1986). The exact site or sites of action are not known. Biochemical pathways are closely interrelated, and sufficient knowledge of all the control and regulatory mechanisms does not exist (Heath, 1988). The previous criteria document summarized the overall processes controlling plant response to O_3.

> "The response of vascular plants to O_3 may be viewed as the culmination of a sequence of physical, biochemical, and physiological events. Ozone in the ambient air does not impair processes or performance, only the O_3 that diffuses

into the plant. An effect will occur only if a sufficient amount of O_3 reaches the sensitive cellular sites within the leaf. The O_3 diffuses from the ambient air into the leaf through the stomata, which can exert some control on O_3 uptake to the active sites within the leaf. Ozone injury will not occur if (1) the rate of O_3 uptake is sufficiently small that the plant is able to detoxify or metabolize O_3 or its metabolites; or (2) the plant is able to repair or compensate for the O_3 impacts (Tingey and Taylor, 1982). The uptake and movement of O_3 to the sensitive cellular sites are subject to various physiological and biochemical controls" (U.S. Environmental Protection Agency, 1986).

Responses to O_3 exposure that have been measured include reduced net CO_2 exchange rate (photosynthesis minus respiration), increased leaf/needle senescence, increased production of ethylene, and changes in allocation patterns. Overall understanding of the response of plants to O_3 has been refined since the last criteria document (U.S. Environmental Protection Agency, 1986). Increased emphasis has been placed on the response of the process of photosynthesis to O_3, on identification of detoxification mechanisms, and on changes in biomass (sugar and carbohydrate) allocation.

As indicated above, entry of O_3 into leaves involves the gas-phase external to the plant and the liquid-phase within the leaf cells. A precondition for O_3 to affect plant function is that it be absorbed into the tissues. Ozone uptake will be divided into two components: adsorption to surfaces and absorption into tissues. Adsorption will affect surface materials (e.g., cuticles) but have little direct affect on physiological processes, whereas O_3 absorption can affect physiological function if O_3 is not detoxified. In the following section, the processes that control movement of O_3 into the plant canopy and then into the leaf will be examined.

5.3.2 Ozone Uptake

Uptake of O_3 in a plant canopy is a complex process involving adsorption of O_3 to surfaces (stems, leaves, and soil) and absorption into tissues, primarily in the leaves (Figure 5-2). Movement of O_3 from the atmosphere to the leaf involves micrometeorological processes (especially wind) and the architecture of the canopy (including the leaves). Within the canopy, O_3 can be scavenged by chemicals in the atmosphere (Kotzias et al., 1990; Gäb et al., 1985; Becker et al., 1990; Yokouchi and Ambe, 1985; Bors et al., 1989; Hewitt et al., 1990); however, the products of these reactions themselves may be phytotoxic (Kotzias et al., 1990; Gäb et al., 1985; Becker et al., 1990; Hewitt et al., 1990). The extent to which these scavenging processes affect O_3 absorption by leaves is not well known. Uptake of O_3 by leaves is controlled, in large part, by the complex of microclimate and canopy architecture, which control movement of O_3 from the atmosphere to the leaf. Leaf conductance is determined by leaf boundary layer conductance and stomatal conductance. In this section, the theoretical and empirical studies on O_3 uptake at the canopy and leaf levels will be examined.

5.3.2.1 Ozone Uptake by Plant Canopies

Integration of O_3 uptake at the stand level requires attention to several levels of organization (Enders et al., 1992; Hosker and Lindberg, 1982) because uptake at this level

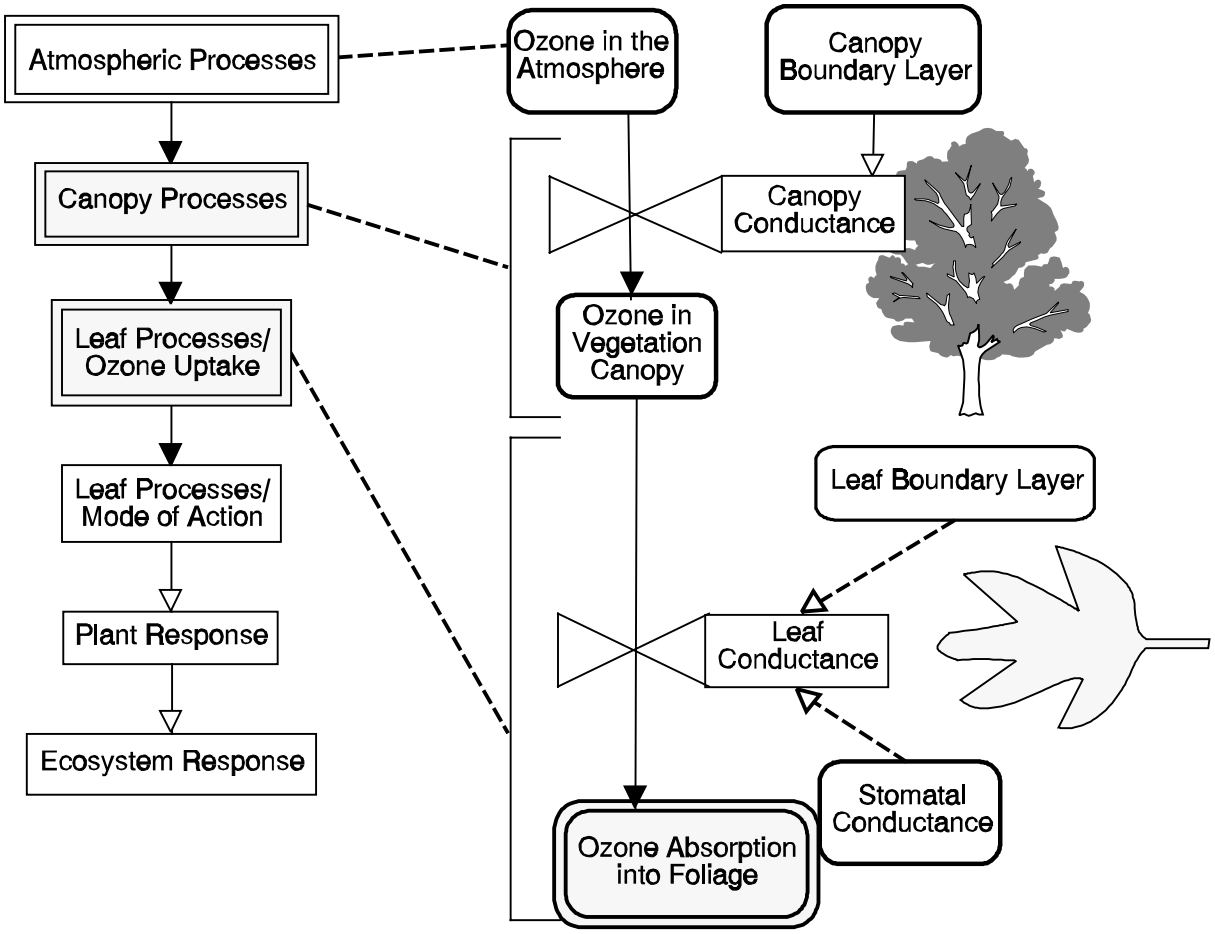

Figure 5-2. Uptake of ozone (O_3) from the atmosphere. Ozone is moved from the atmosphere above the canopy boundary layer into the canopy primarily by turbulent flow of air. Canopy conductance, controlled by the complexity of the canopy architecture and the wide distribution within the canopy, is a measure of the ease with which gases move into the canopy. Within the canopy, O_3 can be adsorbed by surfaces as well as being absorbed into the foliage. Foliage absorption is controlled by two conductances, leaf boundary layer and stomatal, which together determine leaf conductance. The solid black arrows denote O_3 flow; dotted arrows indicate processes affecting uptake or response to O_3. Boxes at the left with double borders are those processes described in the figure. The rounded box with a double border is the end of pathway on this figure.

includes not only uptake by leaves but also adsorption by stems, the soil, and other structures with which O_3 can react. Although the actual pathway, and therefore conductance, will vary within the canopy, depending on position and wind profile, an integrated average conductance is frequently used to describe canopy conductance (Monteith and Unsworth, 1990). For most tree species, canopy conductance tends toward high values, whereas, for crops, it tends to be low.

Two general approaches have been used to estimate O_3 uptake by a plant stand: (1) measurement of gradients over the canopy using micrometeorological methods and (2) simulation of canopy conductance. The results of the two methods generally are different because the micrometeorological techniques include O_3 uptake by all surfaces, whereas simulation accounts only for O_3 absorbed by the surfaces simulated, primarily the foliage.

Two micrometeorological methods, (1) Bowen ratio and (2) eddy correlation, have been used to calculate canopy O_3 uptake. The Bowen ratio assumes a constant relationship between heat and water vapor fluxes (i.e., sensible and latent heat), then calculates O_3 uptake assuming a constant relation between water vapor and O_3 fluxes (Leuning et al., 1979a). The eddy correlation technique requires more elaborate instrumentation for measurement of variation in temperature, water vapor, and O_3 concentration over time and has stringent site requirements (Wesely et al., 1978).

Wesely et al. (1978), using eddy correlation, found a strong diurnal variation in the deposition velocity (the inverse of canopy conductance) and O_3 flux over a corn canopy. They also found evidence that 20 to 50% of the flux was to the soil and to the surface of the canopy. Ozone flux to a dead corn canopy also had a diurnal variation, but a lower magnitude, probably reflecting the absence of uptake through the stomata. Single time measures of deposition velocity, or canopy resistance, have been taken in a Gulf Coast pine forest (54 s m^{-1}; Lenschow et al., 1982) and in a New Jersey pine forest (120 and 300 s cm^{-1}; Greenhut, 1983). Ozone uptake in a maple forest varied diurnally in a pattern explainable by variation in leaf conductance and O_3 concentration (Fuentes et al., 1992). Ozone flux below the tree canopy at 10 m was about 10% of the flux above the canopy at 33 m. Measurements in specially constructed chambers showed that O_3 uptake, as well as photosynthesis, could occur when the foliage was wet (Fuentes and Gillespie, 1992). The fact that wet leaves could take up significant CO_2 is evidence that the stomata were not blocked by the water on the leaf surface. This result is counter to assumptions made in earlier work (Baldocchi et al., 1987) in which water on the surface of the leaf was presumed to interfere with O_3 uptake.

Simulation of canopy conductance requires scaling uptake from individual leaves to individual trees to that of a stand using a combination of canopy models (one for each species) and a stand model to handle interactions among individuals. Several assumptions are required for this approach: the primary sink for O_3 is the foliage, variation in stomatal conductance can be simulated through the canopy using either direct measurements or models, and canopy and plant models adequately simulate response when competition is occurring.

Leuning et al. (1979a,b) used a simple model to estimate canopy uptake in corn (*Zea mays*) and tobacco. Comparison of the results of these simulations with estimates using the Bowen ratio technique indicated that about 50% of the O_3 absorbed by the stands entered the leaves. Baldocchi et al. (1987) presented a model for canopy uptake of O_3 that incorporated stomatal function, some aspects of canopy architecture, and soil uptake. The results of the simulation of O_3 uptake by a corn canopy correlated well with estimations using the Bowen ratio, but tended to overestimate the magnitude. These authors point out that results of model simulation are quite sensitive to the assumptions used. As part of a series of simulations, Reich et al. (1990) explored the effects of different O_3 exposures (daily average O_3 concentrations of 0.035, 0.05, 0.065, and 0.080 ppm) on canopy carbon gain in a mixed oak-maple forest. Depending on the response function and O_3 exposure used, reductions in carbon gain were between 5 and 60%. An important result of these simulations is that the effect of O_3 was strongest in the upper layer of the canopy, where most of the photosynthesis occurred. Although all these simulations provide some interesting insights into how

O_3 uptake (and response) varies with time and exposure, data for validating the models are still needed.

Grünhage and Jäger (1994a,b), using information gathered from a micrometeorological study of O_3 flux observations above a natural grassland in Germany, developed a mathematical model to describe the flux and to estimate the potential injury to the grassland. The aim of the paper was to explain how both vertical flux and stomatal conductance changed during the day and influenced the uptake of air pollutants. For this reason, under ambient conditions, exposures cannot be expressed as a simple function of the pollutant concentration in air.

In summary, O_3 uptake (absorption to surfaces and absorption by tissues) by plant canopies has been measured only a few times. The results are consistent with the hypothesis that stomatal conductance plays a major role in the process. Modeling of O_3 absorption by leaves provides a means of assessing the understanding of the processes controlling O_3 absorption. Combining direct measurements over canopies with modeling will provide a means for assessing the dynamics of O_3 uptake in a canopy.

5.3.2.2 Ozone Absorption by Leaves

The importance of stomatal conductance for the regulation of O_3 uptake by a canopy has been hypothesized for some time (Heck et al., 1966; Rich et al., 1970). Uptake of O_3 by leaves is controlled primarily by stomatal conductance, which varies as a function of stomatal aperture (Figure 5-3). Kerstiens and Lendzian (1989) found that the permeability of cuticles by O_3 from several species was about 0.00001 that of open stomata. Movement of guard cells, which control stomatal opening, are affected by a variety of environmental and internal factors, including light, humidity, CO_2 concentration, and water status of the plant (Zeiger et al., 1987; Kearns and Assmann, 1993). Air pollutants, including O_3, also may affect stomatal function (U.S. Environmental Protection Agency, 1986). The pattern of diurnal stomatal conductance is produced by the integrated response of guard cells to a variety of factors.

As the primary "gate keepers" for gas exchange between the atmosphere and the leaf, stomata perform the vital function of controlling the movement of gases, including air pollutants such as O_3, to and from the leaf. The complexity of the response of stomata to environmental (microclimatic and edaphic) factors is indicated by the large amount of research on stomatal physiology and response to changing conditions (for reviews, see Zeiger et al., 1987; Schulze and Hall, 1982) and on developing models to simulate stomatal response (Avissar et al., 1985; Ball et al., 1987; Collatz et al., 1991; Eamus and Murray, 1991; Friend, 1991; Gross et al., 1991; Johnson et al., 1991; Küppers and Schulze, 1985). The magnitude and diurnal pattern of stomatal conductance depends on both internal, species-specific factors and on the external environment, including soil fertility and nutrient availability, as well as microclimate (Schulze and Hall, 1982; Beadle et al., 1985a,b). Mid-day stomatal closure is observed frequently under conditions of high temperature and low water availability (Helms, 1970; Tenhunen et al., 1980; Weber and Gates, 1990). As an example of the variability in diurnal gas exchange, Tenhunen et al. (1980) present nine graphs of diurnal photosynthesis for apricot (*Prunus armeniaca*) measured from July to September 1976. Although there is a general pattern of increase in the morning and of decline in the evening, the path of photosynthesis and conductance are quite different among

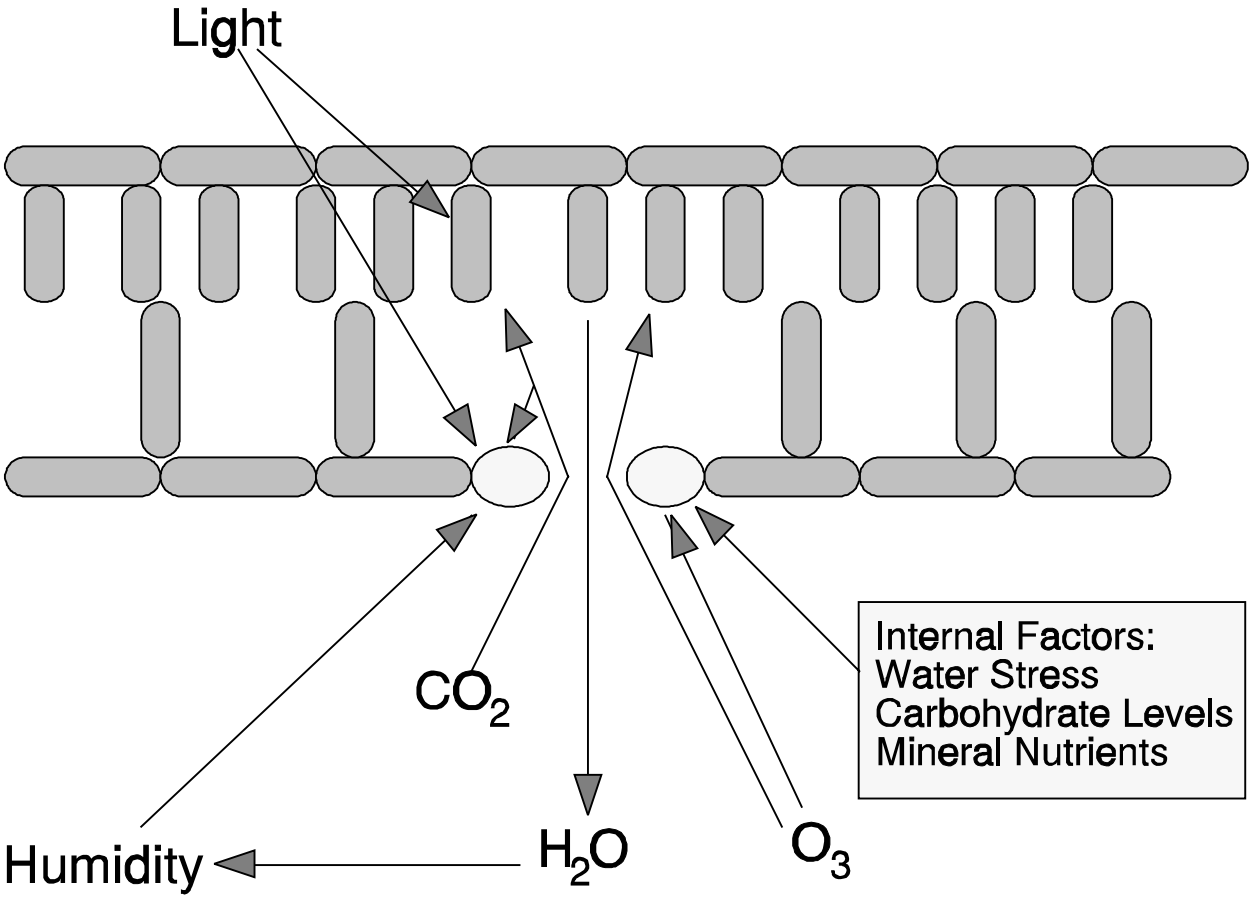

Figure 5-3. Movement of gases into and out of leaves is controlled primarily by the stomata (small openings in the leaf surface whose aperture is controlled by two guard cells). Guard cells respond to a number of external and internal factors, including light, humidity, carbon dioxide (CO_2), and water stress. In general, the stomata open in response to light and increasing temperature and close in response to decreasing humidity, increased CO_2, and increasing water stress. They also may close in response to air pollutants, such as ozone.

of days. The inherent variability in stomatal opening makes using a set time period for O_3 exposure problematic. This variability makes determining the effects of a given diurnal O_3 exposure pattern difficult without reference to physiological, meteorological, and edaphic information, as well as to the sensitivity of individual species exposed.

To be absorbed, O_3 must be present in the atmosphere surrounding the leaf and the stomata must be open. Any factor that affects stomatal opening affects O_3 absorption (Figure 5-3). Under drought conditions, when stomatal conductance is reduced, the relative effect of O_3 is less when compared with well-watered controls (Tingey and Hogsett, 1985; Flagler et al., 1987; Temple et al., 1993, also see Section 5.4). Low humidity has been shown to modify plant response to O_3 (McLaughlin and Taylor, 1981), presumably due to reduced O_3 absorption (Wieser and Havranek, 1993).

To calculate O_3 absorption, some estimate of the internal O_3 concentration must be made. In earlier work, a finite O_3 concentration was assumed to exist in the intercellular air space of the leaf (Bennett et al., 1973; Tingey and Taylor, 1982; Lange et al., 1989). Estimating this concentration is difficult because the rate of O_3 absorption into the leaf must be known. Recently Laisk et al. (1989) presented evidence that this concentration is near zero, a result that is consistent with the highly reactive nature of O_3. Further studies on other species must be made to test the hypothesis that internal O_3 concentration is negligible in leaves.

The other component of absorption, O_3 concentration outside the leaf, may vary greatly with time of day and season (Chapter 4). Data on the effect of variations in O_3 profile (from constant concentrations to equal daily peaks to variable [episodic] peaks), based on greenhouse and OTC chamber studies using simulated exposures, suggest that those profiles that have periodic high concentrations have a greater effect than those with low peaks even though the exposure is equivalent (Hogsett et al., 1985a; Musselman et al., 1986b; see Section 5.6). Taylor and Hanson (1992) show how variations in conductance can affect O_3 absorption and conclude that conductances in and near the leaf surface have a major influence on absorption of O_3. Figure 5-4 shows a simulation of the effect of diurnal variation in stomatal conductance and O_3 concentration on the O_3 absorbed into the leaf. Amiro and Gillespie (1985) found that cumulative O_3 absorption correlated with visible injury in soybean. Weber et al. (1993) found that rate of uptake may play an important role in the response of ponderosa pine (*Pinus ponderosa*). The roles of cumulative uptake versus uptake rate have not been clarified and need further study.

Absorption of O_3 by leaves depends on variations in both stomatal conductance and O_3 concentration. The highly reactive nature of O_3 makes measuring its absorption difficult; therefore, models of stomatal conductance are used, along with O_3 concentrations, to estimate O_3 absorption. The relative importance of absorption rate versus cumulative absorption is not known at present.

5.3.3 Resistance Mechanisms

Resistance mechanisms can be divided into two types: (1) exclusion from sensitive tissue and (2) detoxification near or in sensitive tissue. For leaves, the former involve response and cuticles, and the latter involve various potential chemical and biochemical reactions that chemically reduce O_3 in a controlled manner. Although these systems potentially provide protection against O_3 injury to tissue physiology, they come at some cost, either in the reduction in photosynthesis, in the case of stomatal closure, or in carbohydrate used to produce detoxification systems.

Injury to leaf and needle cuticles does not appear to have a major effect on leaf function, based on the inconsistent data. Barnes et al. (1988a) found that O_3 exposure could damage leaf cuticles; however, Lütz et al. (1990) found no consistent changes in cuticle structure in Norway spruce (*Picea abies*).

5.3.3.1 Stomatal Limitation

As noted above, stomata can be affected by a wide variety of environmental factors (Section 5.3.2.2), by occurrences of stress (Section 5.4), and by age. In addition,

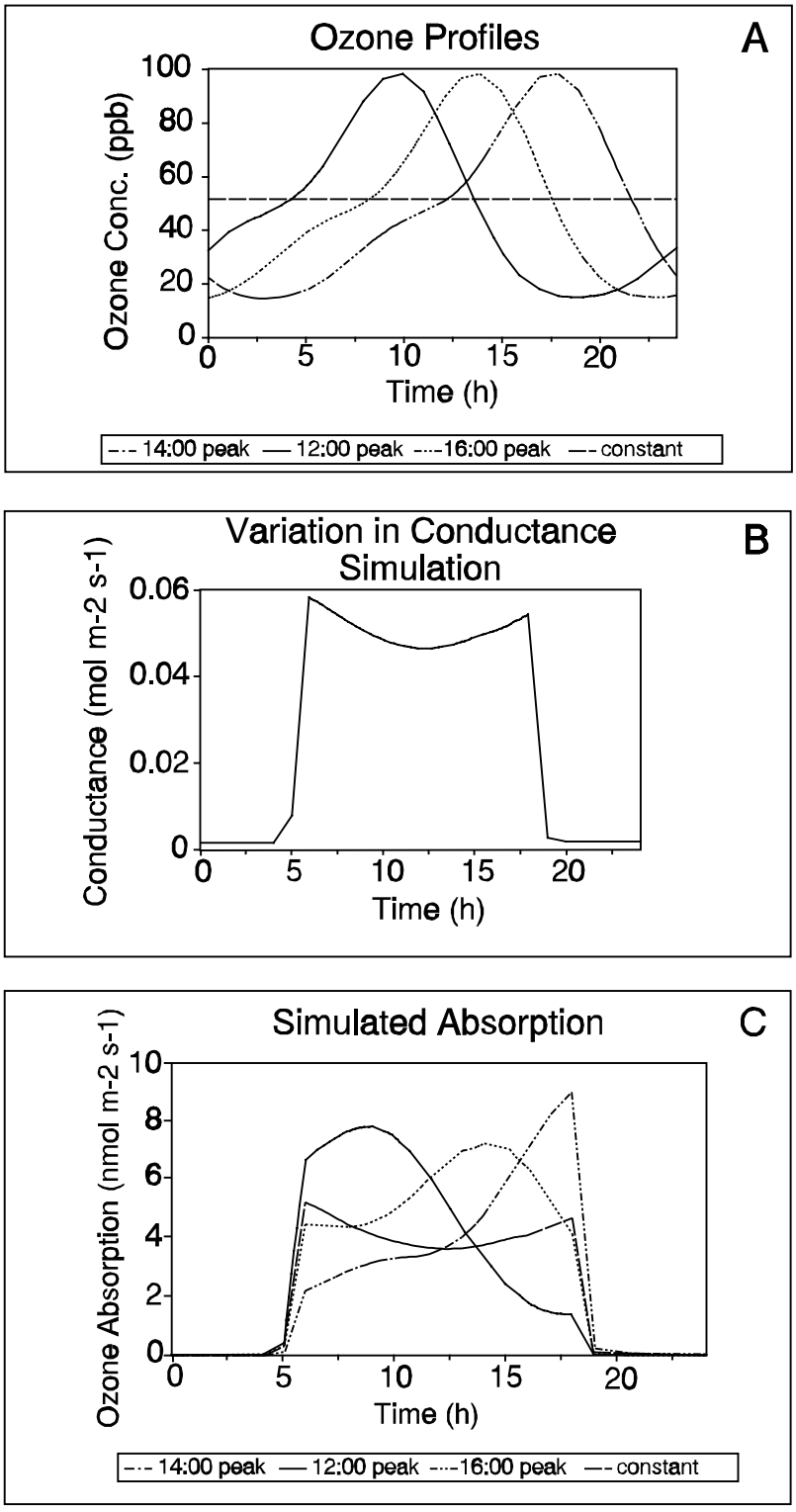

Figure 5-4. Simulation of the effects of diurnal variation in stomatal aperture and in ozone (O_3) concentration on O_3 uptake: (A) diurnal O_3 concentrations, (B) simulated conductance, and (C) O_3 uptake.

stomatal response can vary among species. These multiple interactions make accurate prediction of uptake under field conditions difficult. Some early research showed a decrease in leaf conductance (Figure 5-3), with O_3 exposure implying a direct effect of O_3 on stomatal conductance (U.S. Environmental Protection Agency, 1986). In studies at high O_3 concentrations (>0.3 ppm), stomatal response was rapid (Moldau et al., 1990). In other studies, reduction in conductance in response to O_3 required hours to days of exposure (Dann and Pell, 1989; Weber et al., 1993). Several studies have shown that discrimination against C_{13} in C_3 plants decreases with O_3 fumigation (Okano et al., 1985; Martin et al., 1988; Greitner and Winner, 1988; Saurer et al., 1991; Matyssek et al., 1992). These data are consistent with an increased restriction of diffusion of CO_2 into the leaf (Farquhar et al., 1989). However, Matyssek et al. (1992) and Saurer et al. (1991) found that internal CO_2 increased with O_3 exposure, and that water-use efficiency decreased, both the opposite of expectation, indicating that photosynthesis decreased relatively more than conductance. Although stomata limit O_3 uptake and may respond directly to high O_3 concentrations (e.g., >0.2 ppm, U.S. Environmental Protection Agency, 1986; Moldau et al., 1990), the relative importance of this response, compared to indirect effects induced by reductions in photosynthetic performance, has not been fully assessed.

5.3.3.2 Detoxification

When O_3 enters a cell, several highly reactive compounds can be produced (e.g., superoxide, free radicals, peroxides) (Heath, 1988). The effects of these compounds depends on their reactivity, mobility, and half-life. For detoxification to occur, oxidant and antioxidant must occur proximately. In addition, the rate of production of antioxidant must be a significant portion of the rate of oxidant entry into the system for effective detoxification to occur. Two general kinds of detoxification systems have been reported in plants: (1) those that utilize reductants (e.g., ascorbate) to reduce O_3 and (2) those that utilize enzymes (superoxide dismutase). In either case, excess oxidizing power is dissipated in a controlled manner, effectively protecting the tissue from damage. These protective systems probably developed in response to photooxidation, which can occur, for example, at low temperatures (Powles, 1984).

Several antioxidants have been reported, the most studied being ascorbate and glutathione (GSH). Much of this work has occurred since the 1986 criteria document (U.S. Environmental Protection Agency, 1986). Alscher and Amthor (1988) reviewed the literature in this area. In the chloroplast, the process requires dihydronicotinamide adenine dinucleotide phosphate and may be a cause for the transient reduction in photosynthesis observed in some studies (Alscher and Amthor, 1988).

Evidence for the participation of antioxidants in protecting cells from O_3 injury is primarily indirect (i.e., changes in levels of antioxidants or of associated enzymes). In red spruce (*Picea rubens*), GSH levels increased in year-old needles in response to O_3, but not in current-year needles (Hausladen et al., 1990; Madamanchi et al., 1991). Dohmen et al. (1990) found increased concentrations of reduced glutathione in Norway spruce in response to long-term O_3 fumigation. In a poplar hybrid (*Populus maximowiczii* x *P. trichocarpa*), total GSH increased with O_3 fumigation; however, the ratio of reduced forms to oxidized forms declined, indicating that oxidation of GSH possibly was stimulated by O_3 (Gupta et al., 1991). Mehlhorn et al. (1986) found that both GSH and ascorbic acid (AH_2) increased with O_3 fumigation in silver fir (*Abies alba*) and Norway spruce. The potential for AH_2 to protect cells from O_3 damage was explored by Chameides (1989), who concluded that such protection

was possible if AH_2 occurred in the apoplast at sufficient concentrations and production rates; however, experimental data are needed to test this hypothesis.

The response of enzymes involved in detoxification is not clear. Activities of enzymes involved in antioxidant production increased in response to O_3 in one study (Price et al., 1990); however, in several others, no effect was found (Madamanchi et al., 1992; Pitcher et al., 1991; Anderson et al., 1992; Nast et al., 1993). Activity of superoxide dismutase (SOD), an enzyme that can reduce one of the products of O_3 interaction with the cytoplasm, can be increased by O_3 fumigation (Alscher and Amthor, 1988; Gupta et al., 1991). There are both cytosolic and chloroplastic forms of this enzyme, but the role the different forms play in detoxification of O_3 is not clear. Teppermann and Dunsmuir (1990) and Pitcher et al. (1991) found that increased production of SOD had no effect on resistance to O_3 in tobacco.

The extent to which these detoxification systems can protect tissue from O_3 damage is unknown. However, "if plants have detoxification mechanisms which are kinetically limited, the rate of O_3 uptake may be important, so that even an integrated absorbed dose may be insufficient to account for observed responses" (Cape and Unsworth, 1988). Potential rates of detoxification for given tissues are needed to estimate the importance of these systems to overall O_3 response. In addition, the sites in which the detoxification systems occur need to be identified.

5.3.4 Physiological Effects of Ozone

The initial reactions of O_3 with cellular constituents are not known. The high reactivity and nonspecificity of O_3 reactions, coupled with the absence of a useful isotopic tag for O_3, make studies of the initial reactions difficult at best. The data on changes in biochemical function resulting from O_3 exposure probably represent effects one or more steps beyond the initial reactions. Nonetheless, data is available that indicate the wide range of cellular processes that can be affected by O_3.

Ozone that has not been neutralized by one of the detoxification systems (Figure 5-5) acts first at the biochemical level to impair the functioning of various cellular processes (Tingey and Taylor, 1982; U.S. Environmental Protection Agency, 1986). The result of these impairments are reflected in integrated changes in enzyme activities, membrane function, and energy utilization (Queiroz, 1988). Several related papers have shown that the activity of the primary carboxylating enzyme (RuBP-carboxylase) is reduced by O_3 exposures in the range of those measured at some sites (Dann and Pell, 1989; Enyedi et al., 1992; Pell et al., 1992; Landry and Pell, 1993). Membrane injury has been found in some experiments using acute levels of O_3 (Heath, 1988). Chronic exposure can lead to changes in lipid composition and in cold resistance (Brown et al., 1987; Davison et al., 1988; DeHayes et al., 1991; Lucas et al., 1988; Wolfenden and Wellburn, 1991). Recently, Floyd et al. (1989) have shown that O_3 can affect nuclear deoxyribonucleic acid (DNA).

Changes in the in vivo concentrations of various growth regulators in response to O_3 exposure could have important consequences for plant function. However, the effects of O_3 on levels and activities of growth regulators have not been studied extensively. Ozone has been shown to stimulate ethylene production, and inhibitors of ethylene production have been found to reduce the effects of O_3 in short-term experiments (Pell and Puente, 1986; Rodecap and Tingey, 1986; Taylor et al., 1988b; Mehlhorn et al., 1991; Telewski, 1992; Langebartels et al., 1991; Mehlhorn and Wellburn, 1987; Kargiolaki et al., 1991; Reddy

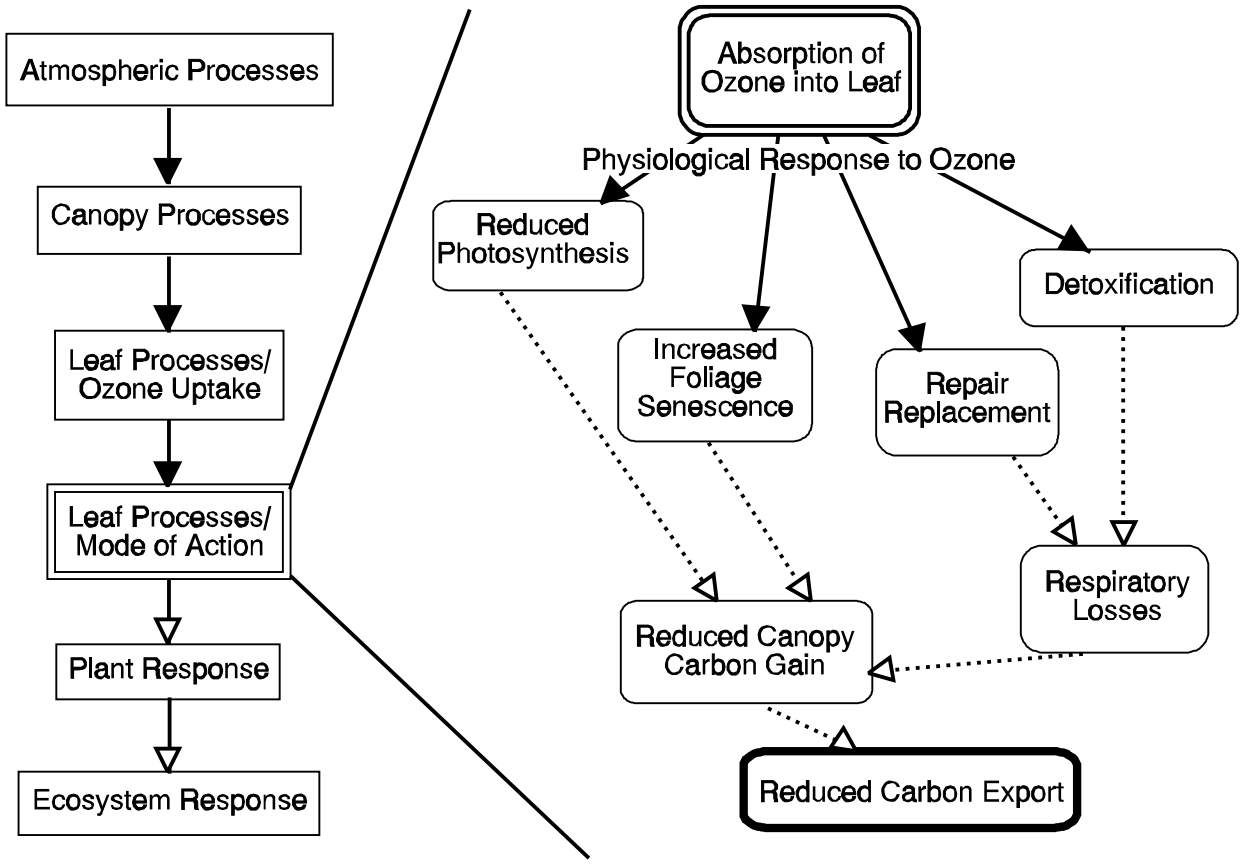

Figure 5-5. Effects of ozone (O_3) absorption into a leaf. Once inside the leaf, O_3 can have a number of effects, all of which affect carbohydrate production and utilization. Reduced photosynthesis, increased leaf senescence, production of detoxification systems, and increased respiration (both maintenance and growth) reduce the amount of carbohydrate available for allocation. Compensation through production of new leaves, for instance, can counter some or all of these effects, depending on the O_3 exposure, the physiological state of the plant, and the species. Integration of these processes leads to changes in the amount of carbohydrate available for allocation from the canopy. Solid black arrows denote O_3 flow, and dotted arrows show the cascade of effects of O_3 absorption on leaf function. Boxes at the left and at the top with double borders indicate leaf processes; the box at the bottom with a dark border indicates the impact.

et al., 1993). Ethylene is produced during ripening of fruit, during periods of stress, and during senescence (Abeles et al., 1992). Increased levels of ethylene in the leaves could play a role in the early senescence of foliage. In some cases, there is a correlation between ethylene production and O_3 sensitivity; however, the relationship is complex and makes use of ethylene production as an index of sensitivity problematic (Pell, 1988).

Abscisic acid (ABA) plays an important role in stomatal function (Davies et al., 1980). Atkinson et al. (1991) found that stomata from O_3 fumigated leaves were less sensitive to ABA than control leaves. Maier-Maercker and Koch (1991a,b; 1992a,b) found that exposure to ambient pollutants, including O_3 and SO_2, caused histological changes in guard cells and resulted in some loss in stomatal control. Results from studies on European white birch (*Betula pendula*) also indicate some change in stomatal function (Matyssek et al., 1992). These data could explain the observation that stomatal function may be impaired by long-term O_3 exposure (Walmsley et al., 1980). Kobriger et al. (1984) found no effect of O_3 on whole-leaf content of ABA, but changes in compartmentation could not be ruled out.

Physiological effects of O_3 uptake are manifest in two ways: (1) reduced net photosynthesis and (2) increased senescence (Figure 5-5). Both decreased photosynthesis and increased leaf senescence result in the loss of capacity for plants to form carbohydrates, thereby potentially having a major impact on the growth of the plant (Figure 5-6). The exact response of a given individual will depend on its ability to compensate for O_3 injury.

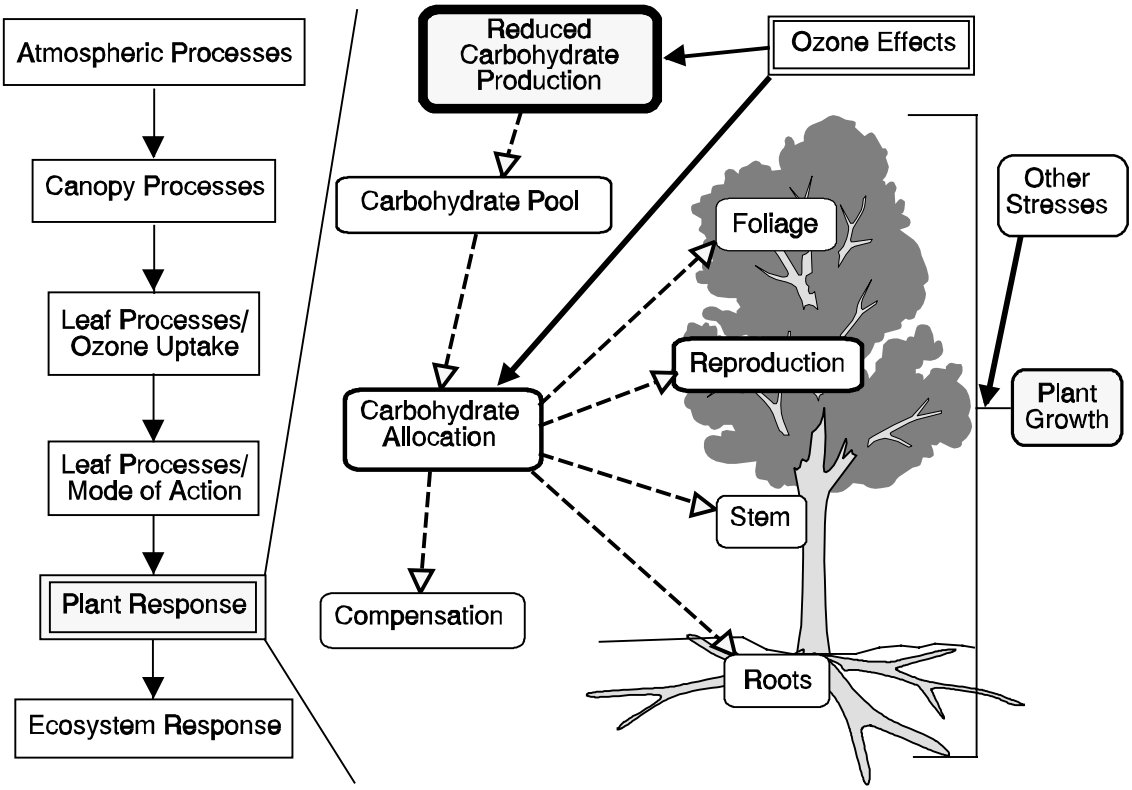

Figure 5-6. Effect of ozone (O_3) on plant function and growth. Reduction in carbohydrate allocation affects the pool of carbohydrates available for growth. Changes in relative growth rate of various organs as a function of O_3 exposure suggest that allocation patterns of carbohydrate are affected. Solid black arrows denote where O_3 absorption affects the allocation processes of the plant; dotted arrows show the cascade to plant growth. Boxes with dark borders indicate site of impact. The box with a double border, at left, indicates the location of response.

Ozone-induced reduction in net photosynthesis has been known for some time (U.S. Environmental Protection Agency, 1986). Changes in stomatal conductance, photosynthetic capacity, carbohydrate allocation, and respiration have been documented. The relationship between O_3 exposure and photosynthesis is not well known. Photosynthesis provides plants with the energy and structural building blocks necessary for their existence. The photosynthetic capacity of a plant is an important aspect of plant response to stresses in natural environments and is strongly associated with leaf nitrogen content and with water movement. Both resources are essential if the process is to occur and involves the allocation of carbohydrates from the leaves to the roots for nitrogen acquisition and water uptake. Leaf photosynthetic capacity is also age dependent. As the plant grows, the canopy structure changes altering the amount and angle of light hitting a leaf. Allocation of carbohydrates and nutrients to new leaves is especially important in stimulating growth production (Pearcy et al., 1987). Reductions in photosynthesis are likely to be accompanied by a shift in growth pattern that favors shoots and by an increase or decrease in leaf life span (Winner and Atkinson, 1986). Therefore, alteration of the processes of photosynthesis and carbohydrate allocation affects plant response to stresses such as O_3. Reduction in photosynthesis (reduced carbohydrate formation and allocation to leaf repair or to new leaf formation decreases the availability of carbohydrates) potentially alters the normal allocation pattern and, therefore, all aspects of plant growth and reproduction (Figure 5-6). The effects of a reduction in photosynthesis on growth and reproduction was discussed in the previous criteria document (U.S. Environmental Protection Agency, 1986).

Carbohydrate production by a single plant is controlled not only by photosynthetic capacity of the foliage but also by the amount and distribution of that foliage. Stow et al. (1992) and Kress et al. (1992) found that O_3 exposure affected needle retention in loblolly pine (*Pinus taeda*). Similar data have been reported for slash pine (*Pinus elliotti*) (Byres et al., 1992a). Keller (1988) and Matyssek et al. (1993a,b) reported increased senescence with increased O_3 exposure in trembling aspen, as did Wiltshire et al. (1993) in apple (*Malus spp*). Replacement of injured leaf tissue has been reported for some species when they are exposed to low O_3 concentrations (Held et al., 1991; Temple et al., 1993). Temple et al. (1993) also found increased photosynthetic capacity of new needles in O_3 treatments compared to controls.

Few direct effects of O_3 have been found outside leaves. Kargiolaki et al. (1991) found that intumescences (lesions) appear on stems of three species of poplar (*Populus*) after 72 days of O_3 fumigation (70 to 80 ppb). Ozone probably enters the stem through the lenticles that occur on the surface of the stem and allow direct exchange of gases between the stem and the air. The consequence of this response to O_3 is not clear; however, it may be related to the reduction in phloem transport rate observed in loblolly pine (Spence et al., 1990).

5.3.4.1 Carbohydrate Production and Allocation

The importance of photosynthesis and carbohydrate allocation in plant growth and reproduction has been pointed out previously. The patterns of carbohydrate allocation directly affect growth rate. Plants require a balance of resources to maintain optimal growth; however, in natural environments optimal conditions seldom occur. Therefore, some plants compensate for differences in resource availability and for environmental stresses. They do this by changing the way they allocate carbohydrates (Chapin et al., 1987). Each response to stress affects the availability of carbohydrates for allocation from the leaves (Figure 5-5).

The carbohydrate pool is affected both by a reduction in the carbohydrate produced and by a shift of carbohydrate to repair and replacement processes. The effect is particularly noticeable in the roots where O_3 exposure significantly reduces available carbohydrate (Andersen et al., 1991; Andersen and Rygiewicz, 1991). Effects on leaf and needle carbohydrate content have varied from a reduction (Barnes et al., 1990b; Miller et al., 1989c) to no effect (Alscher et al., 1989) to an increase (Luethy-Krause and Landolt, 1990). Cooley and Manning (1987) reviewed the literature on carbohydrate partitioning and noted that "storage organs ... are most affected by O_3-induced partitioning changes when O_3 concentrations are in the range commonly observed in polluted ambient air." Friend and Tomlinson (1992) found that O_3 exposure increased retention of ^{14}C-labeled photosynthate in needles of loblolly pine, and modified the distribution of labels among starch, lipids, and organic acids (Edwards et al., 1992b; Friend et al., 1992).

The above discussion supports the information in the previous criteria document (U.S. Environmental Protection Agency, 1986), which pointed out that roots usually were affected more by O_3 exposures than were the shoots. Studies by Miller et al. (1969), Tingey et al. (1976b), McLaughlin et al. (1982), and Price and Treshow (1972) were cited in support of this view. Miller et al. (1969) noted that reduction in photosynthesis was accompanied by decreases in sugar and polysaccharide fraction in injured needles of ponderosa pine seedlings, as well as by altered allocation of carbohydrates. Exposures were for 30 days, 9 h/day, to concentrations of 0.15, 0.30, or 0.40 ppm. These exposures reduced photosynthesis by 10, 70, and 85%, respectively. The observations of Tingey et al. (1976a) indicated that O_3 exposures differentially affected metabolic pools in the roots and tops of ponderosa pine seedlings grown in OTCs. Further, this study indicated that the amounts of soluble sugars, starches, and phenols tended to increase in the tops and decrease in the roots of ponderosa pine seedlings exposed to 0.10 ppm O_3 for 6 h/day for 20 weeks. The sugars and starches stored in the tree roots were significantly less than those in the roots of controls. In another study cited in the 1986 document, McLaughlin et al. (1982) also observed the reduced availability of carbohydrate for allocation to the roots and stated that the result was reduced vigor and enhanced susceptibility of trees to root diseases. Loss of vigor was due to a sequence of events that was associated with exposure to O_3, including premature senescence, loss of older needles, lower gross photosynthetic productivity, and reduced photosynthate (carbohydrates) available for growth and maintenance. Carbon-14 transport patterns also indicated changes in carbon allocation. Older needles were found to be the source of photosynthate for new needle growth in the spring and were storage sinks in the fall. Retention of ^{14}C-photosynthate by foliage and branches of sensitive trees indicated that allocation to the trunks and roots was reduced.

Lost carbohydrate production has effects throughout the plant (Figure 5-6). The roots and associated mycorrhizal fungi are especially susceptible to reduced carbohydrate availability and, quite frequently, show the greatest decline in growth (Adams and O'Neill, 1991; Edwards and Kelly, 1992; McQuattie and Schier, 1992; Meier et al., 1990; Taylor and Davies, 1990). However, in some cases, increased mycorrhizal formation has been reported (Gorissen et al., 1991b; Reich et al., 1985). It might be expected that reduced allocation to roots would affect shoot growth through increased susceptibility to water stress, reduced nutrient availability (Flagler et al., 1987), and reduced production of growth factors (Davies and Zhang, 1991; Letham and Palni, 1983). Effects on production and retention of leaves and needles were described above. Effects on stem growth have been found in tree species (Hogsett et al., 1985b; Mudano et al., 1992; Pathak et al., 1986; Matyssek et al., 1992;

Matyssek et al., 1993b). Changes in canopy density, root/shoot ratio, and stem growth will affect the functioning of the plant and may make plants more susceptible to environmental stresses, such as drought and nutrient limitation, that are characteristic of many ecosystems.

5.3.4.2 Compensation

Compensatory responses occur as plants attempt to minimize the effects of stress. Responses include adjustments to changes in physiological processes (e.g., photosynthetic performance and foliage production) that tend to counteract the effects of O_3 absorption by the leaves. Pell et al. (1994) have reviewed the extensive literature produced in the Response of Plants to Interacting Stresses (ROPIS) experiment (Goldstein and Ferson, 1994). A wide range of compensatory responses have been identified, especially reallocation of resources leading to increased relative growth in the shoot compared to the root (see above). Compensation can take the form of production of new tissue (e.g., leaves) to replace injured tissue or of biochemical shifts, including increased photosynthetic performance in new foliage.

Changes in respiratory rate have been attributed to such repair processes (U.S. Environmental Protection Agency, 1986). Recent studies have found stimulation of dark respiration in Norway spruce (Barnes et al., 1990b; Wallin et al., 1990) and pinto bean (Amthor, 1988; Amthor and Cumming, 1988; Moldau et al., 1991b). Repair of membranes (Sutton and Ting, 1977; Chevrier et al., 1988, 1990) and replacement of impaired enzymes are two probable reasons for increased respiration. Ozone has been shown to increase the adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio, which is consistent with increased respiratory activity (Weidmann et al., 1990; Hampp et al., 1990). As in the case of detoxification, the importance of repair processes in the overall carbohydrate budget of the plant and of their influence of apparent threshold is unknown.

Recovery of photosynthetic performance after O_3 exposure has been noted in some studies. Early work indicated that recovery of photosynthetic capacity could occur after exposure of high concentrations (>0.25 ppm) of O_3 (e.g., Botkin et al., 1971, 1972). Dann and Pell (1989) found that photosynthetic rate, but not Rubisco activity, recovered within a few days in potato (*Solanum tuberosum*) after exposure to 0.2 ppm O_3. In ponderosa pine, photosynthetic rates in O_3 treated needles recovered to that of controls within 40 to 50 days (Weber et al., 1993). To what extent this recovery can offset losses in carbohydrate gain is not known, nor is the mechanism.

Replacement of injured foliage (see Section 5.3.4) is another method to counteract the effects of O_3 exposure. The extent to which increased leaf and needle production and increased photosynthetic performance in the new foliage compensates for O_3 injury is not known.

The importance of various compensatory mechanisms is not sufficiently well known to allow an estimate of the degree to which they might mitigate the effect of O_3. The fact that increases in photosynthesis and in leaf production have been measured indicates that these processes, at least, may be important.

5.3.5 Role of Age and Size Influencing Response to Ozone

Plant age, physiological state, and frequency of exposure play important roles in plant response to O_3. In annual species, effects of O_3 on production will occur through changes in allocation of carbohydrates over the years, resulting in reduced seed production.

In perennial species, plant growth will be affected by reduction in storage of carbohydrates, which may limit growth the following year (carry-over effects). Carry-over effects have been documented in the growth of tree seedlings (Hogsett et al., 1989; Sasek et al., 1991; Temple et al., 1993) and roots (Andersen et al., 1991). Accumulation of these effects will affect survival and ability to reproduce. Data on cumulative effects of multiple years of O_3 exposures have been, for the most part, the result of 2- to 3-year studies.

A tacit assumption in much of the research on O_3 effects on trees is that seedling response to O_3 is a good predictor of large-tree response. This assumption has been necessitated by the difficulty in exposing large trees to O_3 for long periods. Pye (1988) reviewed the problems of extrapolation from seedling/sapling experiments to large trees and noted several areas of difference between seedling/saplings and large trees: (1) microclimate (especially radiation), (2) transport distances, (3) ratio of photosynthetic to respiratory tissue, and (4) potential for storage. Cregg et al. (1989) also argued that these differences in scale can affect growth responses seen. Some studies have indicated that seedlings may be more sensitive (i.e., greater visible injury) than large trees (Kozlowski et al., 1991); however, Samuelson and Edwards (1993) found that leaves on large red oak trees (*Quercus rubra*) are more sensitive than those on seedlings. It is likely that a variety of factors determines sensitivity to O_3, including stomatal function and presence of detoxification systems, so that, in some cases, seedlings will be more sensitive and, in others, large trees will be. Although each of the four differences between small and large trees mentioned above can be supported on theoretical grounds, little direct information is available to evaluate the importance of these differences, especially with respect to O_3.

The microclimate of the canopy of mature trees is quite different from that of seedlings, as is that of a stand of trees compared to a single tree in a field. Light intensity through the multilayer canopy can vary by an order of magnitude or more (Jones, 1992). In addition, gradients of other important microclimatic variables (temperature, humidity, and wind speed) exist within the canopy. These will all affect stomatal conductance, and some (e.g., wind speed) will affect canopy conductance. In addition, leaf development will be affected by these microclimatic variables (especially light intensity), leading to leaves with different physiological capacity and sensitivity to O_3 (Samuelson and Edwards, 1993; Waring and Schlesinger, 1985).

The effect of plant size on transport processes and the subsequent response to O_3 is unknown. The simple fact of greater distance over which transport must occur will affect the timing of response of organs distant from the primary site of O_3 impact, the foliage. Studies using methods that integrate functions over the whole tree could provide useful information. For example, combinations of porometer measurements on foliage and whole-plant water use measured (Schulze et al., 1985) on individuals of different sizes could provide very useful information on the coupling of leaf-level processes to whole-canopy and whole-plant response. Greater evaporative demand in large trees as the result of greater leaf area and different microclimate than in small trees could lead to transient water stress and stomatal closure because of insufficient water transport capacity.

As a tree grows from a seedling to a large tree, the ratio between photosynthesis and respiration declines as a greater portion of the plant tissue becomes nonphotosynthetic. It is reasonable to assume that such a change could result in less resource being available for detoxification and repair as the plant grows. How this change affects the ability of a plant to survive O_3 (or any other stress) is not known. Recently, Samuelson and Edwards (1993) presented data on northern red oak that show O_3 decreased photosynthetic performance more

on lower leaves within the canopy of large trees than on leaves near the top of the canopy (a result apparently counter to the model results of Reich et al., 1990). Seedling photosynthesis was not affected by the same O_3 exposure. A more interesting result of this work is the reduction in total canopy biomass found in large trees exposed to O_3. It is not possible to assess directly the relative importance of reduced photosynthesis versus loss of canopy from these data, but the data do show that differences may exist between large trees and seedlings in their response to O_3. These differences may be due to changes in carbon budgets, stomatal characteristics, microclimate, and flushing patterns that develop as seedlings become trees. The ability of northern red oak seedlings to produce three flushes and thus replace injured foliage may be an important defense mechanism in the seedling stage. The physiological basis of these findings need further investigation.

In evergreen perennial plants, foliage must be maintained from one year to the next, frequently through periods unfavorable to growth. In evergreen species that retain a few to several years of leaves, increased susceptibility to stress (e.g., frost) could further reduce potential canopy photosynthesis in subsequent years (Brown et al., 1987; Davison et al., 1988; DeHayes et al., 1991; Lucas et al., 1988). Fincher (1992) found that O_3 decreased frost tolerance in red spruce in both seedlings and trees; the consequences of this change in seedlings and large trees needs of further study.

The effect of O_3 on storage of carbohydrates in large compared to small trees is not known. Changes in storage could affect the ability of the plant to withstand other stresses or to produce adequate growth during each growing season.

Dendrochronology (tree-ring analysis) provides the opportunity to do retrospective studies over the life of large trees. Reduction in annual radial growth has been found in the southern Sierra Nevada for Jeffrey pine but not for ponderosa pine (Peterson et al., 1987, 1989, 1991; Peterson and Arbaugh, 1988). One difficulty with using tree-ring data to estimate O_3-related effects is that it is not always possible to separate reductions due to O_3 from other effects (e.g., drought).

Development of reliable methods for scaling from small to large trees is crucial to the prediction of the long-term effects of O_3 on forest function. Measurement of the response of different size trees to O_3 could provide useful data on the relative responses of small and large trees. However, problems exist in giving similar exposures to trees of widely different sizes. The most direct method is to fumigate trees over a significant portion of their life span. Time is the primary obstacle to these studies because they would require decades to complete. Whatever methods are used must be based on a good understanding of the physiological changes that occur as trees grow.

5.3.5.1 Summary

In the previous criteria document, it was concluded that the "critical effects, including reduction in photosynthesis and a shift in the assimilation of photosynthate, will lead to reduced biomass, growth, and yield" (U.S. Environmental Protection Agency, 1986). In addition, changes in carbohydrate allocation patterns and effects on foliage were noted as important. Since that report, additional information has been developed, especially on the effects of O_3 on photosynthetic performance. However, at present there is still no clear understanding of the initial biochemical changes resulting within the leaf cells after the entry of O_3 and how these changes interact to produce the observed responses. Much of the earlier research used very high ($\geq$0.25 ppm) O_3 concentrations, which produced what could be characterized as acute responses. More recent research has used lower concentrations, usually

including near ambient (0.04 to 0.06 ppm) O_3 levels, so that the observed responses may be more relevant to field conditions. One characteristic of these more recent data is that a longer exposure (days to weeks, instead of hours) is needed to show a response.

As a result of the research since the last criteria document (U.S. Environmental Protection Agency, 1986), the way in which O_3 exposure reduces photosynthesis, especially its effects on the central carboxylating enzyme (ribulose-6-P-carboxylase/oxygenase), is better understood. The rate of senescence of leaves has been shown to increase as a function of increasing O_3 exposure. At near-ambient exposures, leaf production has been shown to increase in some species, thereby off-setting the increased loss to due senescence. The mechanism of the increase in senescence is not known, hence deserves further study. Finally, the role that changes in allocation of resources play in plant response to O_3 is now better understood. Most studies have shown that allocation of photosynthate to roots is decreased by O_3. In some cases, allocation to leaf production has increased. Whether these changes are driven entirely by changes in carbohydrate availability or are controlled by other factors (e.g., hormones) is not known.

Some potentially significant processes have been investigated since the last criteria document, especially detoxification and compensatory processes. The role of detoxification in providing a level of resistance to O_3 has been investigated; however, it is still not clear to what degree these processes can provide protection against O_3 injury. Data are needed especially on the potential rates of antioxidant production and on the subcellular localization of the antioxidants. Potential rates of antioxidant production are needed to assess whether they are sufficient to detoxify the O_3 as it enters the cell. The localization is needed to assess whether the antioxidants are in a location (cell wall or plasmalemma) that permits contact with the O_3 before it has a chance to damage subcellular systems. Ozone exposure has been shown to decrease cold tolerance of foliage in some species. This response could have a major impact on long-lived evergreen species that retain leaves for several years. Various forms of compensation, especially stimulation of production of new leaves and higher photosynthetic performance of new leaves, have been reported. Although these processes divert resources away from other sinks, compensation may counteract the reduction in canopy carbon fixation caused by O_3. The quantitative importance of these processes is still in need of investigation.

The major problem facing researchers trying to predict long-term O_3 effects on plants is how the plant integrates all of the response to O_3 into the overall response to the environment, including naturally occurring stresses. Little is now known about how response to O_3 changes with increasing age and size. This information is crucial to predicting the long-term consequence of O_3 exposure in forested ecosystems.

5.4 Factors That Modify Plant Response

5.4.1 Modification of Functional and Growth Responses

Plant response to oxidants may be modified by various biological, physical, and chemical factors. Biological factors that modify plant response include those within the plant, as well as those external to the plant. The genetic makeup and the developmental stage play critical roles in the way individual plants respond to O_3 and other external stresses. For example, different varieties or cultivars of a particular species are known to differ greatly in their responses to a given exposure to O_3, whereas the magnitude of the response of a

particular variety, in turn, depends on environmental factors such as temperature and humidity, soil moisture and nutrition, the presence of pests or pathogens, and exposure to other pollutants or agricultural spray chemicals. In other words, response will be dictated by the plant's present and past environmental milieu, which also includes the temporal pattern of exposure and the plant's stage of development. The corollary is also true: exposure to oxidants can modify response to other environmental variables. For example, exposure to O_3 reduces the ability of trees to withstand winter injury caused by exposure to freezing temperatures (Davison et al., 1988) and influences the success of pest infestations (Hain, 1987; Lechowicz, 1987). Hence, both the impact of environmental factors on response to oxidants and the effects of oxidants on responses to environmental factors have to be considered in determining the impact of oxidants on vegetation in the field. These interactions are summarized as the involvement of "other stresses" in the scheme shown in Figure 5-6 (Section 5.3). In the following review, the environmental factors are grouped into three categories: (1) biological (including genetic and developmental components), (2) physical, and (3) chemical.

Runeckles and Chevone (1992) have provided a general review of the interactive effects of environmental factors and O_3. The subject also is treated in a National Acid Precipitation Assessment Program (NAPAP) report (Shriner et al., 1991). The numbers of publications that have appeared since the previous criteria document and supplement vary widely among the different environmental factors reviewed. As a result, in several sections, material covered in these earlier documents has been repeated in order to provide comprehensive coverage and to place new findings into context.

5.4.2 Genetics

The response of an individual plant within a species and at a given age is affected both by its genetic makeup and the environment in which it grows. This section examines the role of genetics in plant response to O_3 and its implication for both managed and natural ecosystems. In addition, major knowledge gaps in the understanding of genetic aspects of O_3 responses are pointed out.

The responses of plants to O_3 are strongly influenced by genetics, as was summarized in the air quality criteria document for O_3 (U.S. Environmental Protection Agency, 1986). Thus, the plants of a given population or family will not respond to O_3 in the same way, even if they are grown in a homogenous environment. This has been demonstrated amply through intraspecific comparisons of O_3 sensitivity as determined by foliar sensitivity of ornamental plants, the aesthetic value of which is decreased by visible foliar injury, and of woody plants that are important components of natural ecosystems (Table 5-2). Ornamental plants and plants growing in wilderness areas, for example, have an intrinsic worth, apart from any economic value related to growth (Tingey et al., 1990). Considerable genetic variation in O_3 sensitivity also has been demonstrated for growth responses of crop plants (Table 5-3). The range of responses displayed for visible foliar injury and growth, biomass, or yield vary from species to species and from study to study. However, it is not uncommon to have genotypes varying from no response to well over 50% leaf area injured or 50% growth or yield reductions in the same study. Additional examples of genetic variation in O_3 response are shown in Figure 5-7 for visible foliar injury and in Figure 5-8 for growth. From Figure 5-7, one can see that, depending on what population has been examined, white ash (*Fraxinus americana*) and green ash (*F. pennsylvanica*) could

Table 5-2. Examples of Intraspecific Variation of Foliar Symptoms in Ozone Response

Species	Genetic Unit[a]	Ozone Concentration	Duration	Range of Response[b]	Reference
Ornamental, Non-woody Plants					
Petunia sp. (Petunia)	Cultivars	400 ppb 4 h/day	4 days	20 to 60% (3)	Elkiey and Ormrod (1979a,b), Elkiey et al. (1979)
Poa pratensis L. (Kentucky bluegrass)	Cultivars	400 ppb 300 ppb	2 h 4 h	0 to 90% (3) 30 to 60% (3)	Murray et al. (1975), Wilton et al. (1972)
Trees					
Acer rubrum L. (Red maple)	Populations	750 ppb 7 h/day	3 days	19 to 34% (2)	Townsend and Dochinger (1974)
Fraxinus americana L. (White ash)	Half-sib families	500 ppb 250 ppb	7.5 h 6 h	0 to 50% (3) 2 to 33% (2)	Karnosky and Steiner (1981), Steiner and Davis (1979)
Fraxinus pennsylvanica Marsh. (Green ash)	Half-sib families	500 ppb 250 ppb	7.5 h 6 h	0 to 40% (3) 2 to 39% (2)	Karnosky and Steiner (1981), Steiner and Davis (1979)
Gleditsia triacanthos L. (Honeylocust)	Cultivars	Ambient	1 growing season	0 to 34% (3)	Karnosky (1981a)
Pinus ponderosa Dougl. ex P. Laws and C. Laws (Ponderosa pine)	Half-sib families	1.5 × ambient	3 growing seasons	0 to 28% (2)	Temple et al. (1992)
Pinus strobus L. (Eastern white pine)	Clones	300 ppb	6 h	0 to 60% (3)	Houston (1974)
Pinus taeda L. (Loblolly pine)	Half-sib families	250 ppb ambient + 60 ppb	8 h 1 growing season	3 to 29% (2) 1 to 42% (1)	Kress et al. (1982a), Adams et al. (1988)
Populus tremuloides Michx. (Trembling aspen)	Clones	200 ppb 150 ppb	3 h 6 h	7 to 56% (1) 10 to 91% (1)	Karnosky (1977), Berrang et al. (1991)

[a]Cultivars = a variety of agricultural or horticultural crops produced by selective breeding or a vegetatively propagated tree selection; Half-sib = seedlings with one parent in common; Clones = vegetatively propagated individual genotypes; and Populations = seedlings derived from a common gene pool.

[b]Range of response is expressed as (1) percentage of leaves showing visible symptoms, (2) percentage of leaf area injured, or (3) percentage from a leaf injury rating scheme.

Table 5-3. Examples of Intraspecific Variation in Growth Responses Following Ozone Exposures

Species	Genetic Unit[a]	Ozone Concentration	Duration	Range of Response[b]	Reference
Crops and Non-woody Plants					
Agrostis capillaris L. (Bentgrass)	Populations	60 ppb	4 weeks	−45 to +20% (2)	Dueck et al. (1987)
Begonia semperflorens Hort. (Bedding begonia)	Cultivars	500 ppb 4 h/day 250 ppb 4 h/day	2 days 4 days	−59 to 0% (2) −16 to +10% (2)	Reinert and Nelson (1979), Reinert and Nelson (1980)
Festuca arundinacea Schreb. (Fescue)	Cultivars	400 ppb 6 h/day	7 days	−53 to −35% (2)	Flagler and Younger (1982)
Lycopersicon esculentum L. (Tomato)	Cultivars	400 ppb 1.5 × ambient	2 h 1 growing season	−50 to −4% (2) −54 to −17% (3)	Reinert and Henderson (1980), Temple (1990a)
Phaseolus vulgaris L. (Snapbean)	Cultivars	60 ppb 7 h 72 ppb 7 h 80 ppb 7 h/day	mean - 44 days mean - 54 days 42 days	−26 to −2% (3) −73 to −44% (3) −68 to −50% (3)	Heck et al. (1988), Temple (1991), Eason and Reinert (1991)
Plantago major L. (Common plantago)	Populations	70 nL/ 1-7 h/day	2 weeks	−24 to 0% (1)	Reiling and Davison (1992a)
Raphanns sativus L. (Radish)	Within cultivar	0.1 µL/ 1-4 h/day 3 days/week	3 weeks	−40 to −5% (2)	Gillespie and Winner (1989)
Silene cucabalus (Bladder campion)	Populations	35 ppb 12 h/day	4 weeks	−75 to −48% (2)	Ernst et al. (1985)
Solanum tuberosum L. (Potato)	Cultivars	150 ppb 6 h/day	8 days	−10 to 0% (2) −40 to 0% (2)	Pell and Pearson (1984), Ormrod et al. (1971)
Spinacia oleracea L. (Spinach)	Cultivars	130 ppb 7 h/day	38 days	−56 to −28% (2)	Heagle et al. (1979a)
Trees and Other Woody Plants					
Acer rubrum L. (Red maple)	Populations	750 ppb 7 h/day	3 days	−36 to −17% (1)	Townsend and Dochinger (1974)
Abies alba Mill. (Silver fir)	Populations	250 ppb 7 h/day	10 days	−18 to +3% (1)	Larsen et al. (1990)
Pinus elliottii Engelm. (Slash pine)	Half-sib families	3 × ambient	3 growing seasons	−20 to 0% (1)	Dean and Johnson (1992)

Table 5-3 (cont'd). Examples of Intraspecific Variation in Growth Responses Following Ozone Exposures

Species	Genetic Unit[a]	Ozone Concentration	Duration	Range of Response[b]	Reference
Pinus taeda L. (Loblolly pine)	Full-sib families	50 ppb 6 h/day 1.9 × ambient	28 days 2 growing seasons	−18 to 0% (1) −19 to 0% (2)	Kress et al. (1982b), Shafer and Heagle (1989)
Pinus taeda L. (Loblolly pine)	Half-sib families	Ambient + 60 ppb 2.5 × ambient 250 ppb	1 growing season 1 growing season 8 h	−27.5 to +3% (2) −19 to −2% (2) −22 to +30% (2)	Adams et al. (1988), Qiu et al. (1992), Winner et al. (1987)
Populus tremuloides Michx. (Trembling aspen)	Clones	26.4 ppm-h ambient	92 days 3 growing seasons	−74 to −5% (2) −24 to −12% (2)	Karnosky et al. (1992a), Wang et al. (1986a,b)
Rhododendron obtusum (Lindl) Planch. (Azalea)	Cultivars	250 ppb - 3 h/day	6 days	−43% to 0% (2)	Sanders and Reinert (1982)

[a]Cultivars = a variety of agricultural or horticultural crops produced by selective breeding or a vegetatively propagated tree selection; Half-sib = seedlings with one parent in common; Clones = vegetatively propagated individual genotypes; and Populations = seedlings derived from a common gene pool.
[b]Range of response is expressed as (1) decrease compared to charcoal-filtered-air control plants in terms of growth, (2) biomass, or (3) yield.

have been classified as either O_3 sensitive or O_3 tolerant. Also noticeable from this figure is the large amount of variation in O_3 tolerance of individual half-sib (one parent in common) families from a given population. From Figure 5-8, the heterogeneity within a given loblolly pine half-sib family in terms of growth is displayed. This variability has some interesting implications. First, because plants of a given species vary widely in their response to O_3 exposure, response relationships generated for a single genotype or small group of genotypes may not represent adequately the responses of the species as a whole (Temple, 1990a). Second, because of the genetic variability and differential fitness extant among different genotypes in a population of plants, O_3 imposes a selective force favoring tolerant genotypes over sensitive ones (Roose et al., 1982; Treshow, 1980). Each of these implications will be discussed in this section.

Mechanisms and Gene Numbers

Little is known about the genetic bases for O_3 resistance mechanisms or about the numbers of genes involved in these mechanisms (Pitelka, 1988). Most O_3 resistance mechanisms involve a physiological cost that will result in decreased growth and productivity of resistant plants grown under O_3 stress. Partial or complete stomatal closure in the presence of O_3 is an example of a mechanism of resistance that has been demonstrated for several plants (Engle and Gabelman, 1966; Thorne and Hanson, 1976; Reich, 1987;

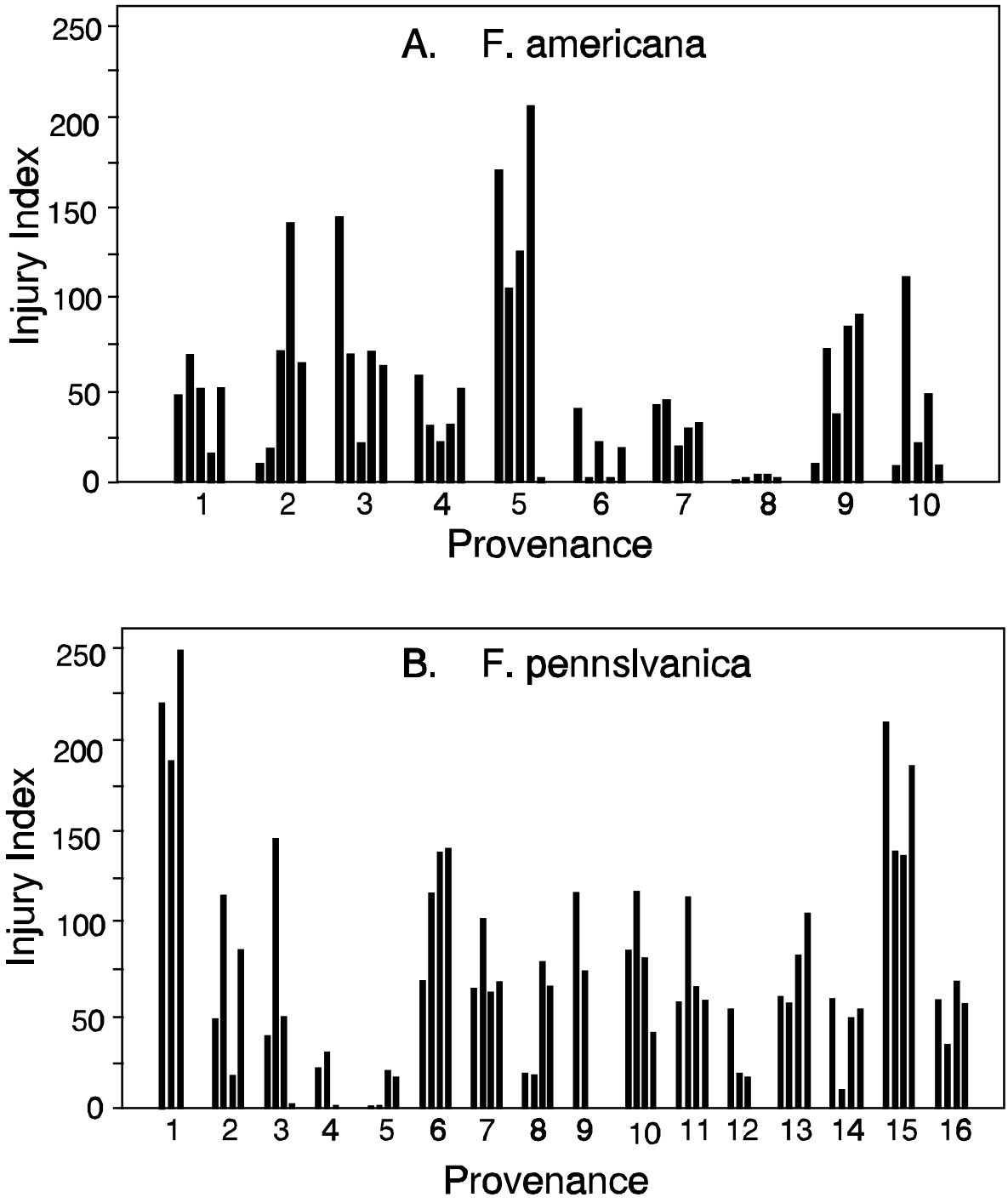

Figure 5-7.

The average injury index for visible foliar injury after exposure of 1-year-old seedlings to 50 pphm ozone for 7.5 h. Each mean shown represents the average of five trees per family. There were either four or five half-sib families for each white ash (*Fraxinus americana *L.) provenance (geographic location) and either three or four families for each green ash (*F. pennsylvanica *Marsh.) provenance. The specifics of the experimental design are reported in Karnosky and Steiner (1981).

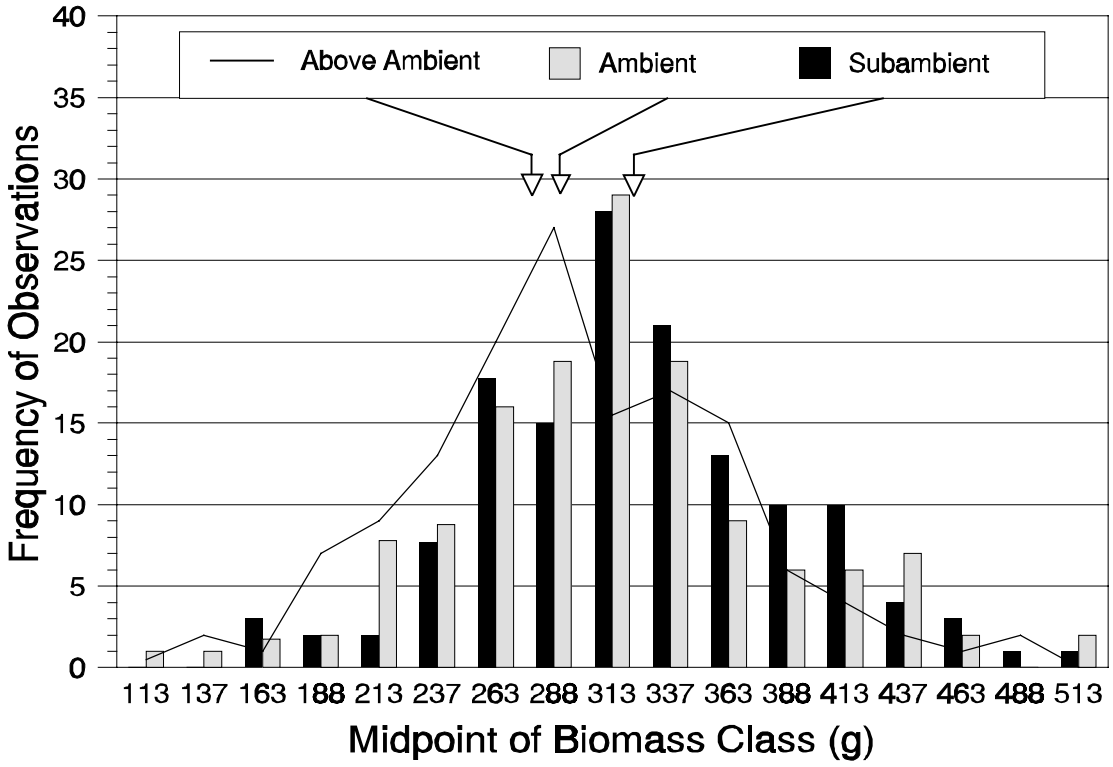

Figure 5-8.

Frequency distribution showing the variability in ozone (O_3) response (midpoint of whole-plant biomass) within one half-sib family of loblolly pine (P. taeda L.) exposed to increasing levels of O_3 under chronic-level field conditions over several growing seasons (Adams et al., 1988). The arrows show the mean response for each of the three O_3 treatments (subambient, ambient, and above-ambient O_3). The specifics of the experimental design are reported by Adams et al. (1988). This figure was developed by Taylor (1994).

Sumizono and Inoue, 1986; Tingey and Taylor, 1982) and that involves a high physiological cost because plants that have reduced stomatal conductivity also will have reduced carbon assimilation for growth (Ehleringer, 1991). Tolerance of internal leaf tissues to O_3 may involve the production of antioxidant defense compounds (Lee and Bennett, 1982; Gupta et al., 1991) or other types of biochemical defense systems. The extent to which these internal tolerance mechanisms have physiological costs associated with them is not yet understood, but it is likely that increased defense compound production, triggered by O_3, will impact the amount of carbon available for growth (Ehleringer, 1991). The genetic regulation of these or other O_3 resistance mechanisms has not yet been characterized thoroughly.

Whether or not O_3 resistance is due to single gene or multi-gene control will affect the rate and extent of resistance development (Roose, 1991). Rapid stomatal closing in the presence of O_3 appears to be under the control of either a single gene or a few genes in onion (*Allium cepa*) (Engle and Gabelman, 1966), some bean (*Phaseolus vulgaris*) cultivars

(cultivated varieties) (Knudson-Butler and Tibbitts, 1979), soybean (Damicone et al., 1987b), and petunia (*Petunia* spp.) (Elkiey and Ormrod, 1979a,b; Elkiey et al., 1979). Generally, resistance mechanisms appear to be more complex (Karnosky, 1989a) and seem to involve multiple gene control as has been demonstrated in tobacco (Aycock, 1972; Huang et al., 1975; Povilaitis, 1967), some bean cultivars (Mebrahtu et al., 1990a,b,c), corn (Cameron, 1975), tall fescue (*Festuca arundinacea*) (Johnston et al., 1983), potato (De Vos et al., 1982; Dragoescu et al., 1987), and loblolly pine (Weir, 1977; Taylor, 1994).

Genetic Implications of Ozone Effects: Managed Ecosystems

Because of the high cost involved in conducting long-term growth studies to determine O_3 effects on plants, only a small proportion of the total number of commercial crop cultivars and important tree seed sources, families, clones, and cultivars have been examined adequately for O_3 sensitivity. Still, a tremendous amount of variation has been found, as was described in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) and in Tables 5-2 and 5-3.

Plant breeders and nurserymen working in locations with high O_3 concentrations have inadvertently developed selections more tolerant to O_3 than those developed in locations with low O_3 exposures (Reinert et al., 1982; Roose et al., 1982). The cultivars Team alfalfa and Kennebec, Pungo, and Katahdin potatoes were developed at the U.S. Department of Agriculture Research Center at Beltsville, where 0.120 ppm O_3 frequently is exceeded (Lefohn and Pinkerton, 1988; Ludwig and Shelar, 1980). These cultivars have proven to be more O_3 tolerant than cultivars developed elsewhere (Reinert et al., 1982). Similarly, cotton (*Gossypium* spp.) and sugar beet (*Beta* spp.) cultivars developed in Southern California, where O_3 levels are among the highest in the country, are more O_3 tolerant than cultivars developed in low O_3 areas (Reinert et al., 1982).

Nurserymen, Christmas tree growers, and seed orchard managers all routinely have discarded pollution-sensitive chlorotic dwarf and tip-burned white pine trees because of their slow growth in areas with high O_3 exposures (Umbach and Davis, 1984). Thus, they have contributed to the selection of more O_3-tolerant commercial forests.

Although these examples suggest that selection of O_3-tolerant genotypes is possible, the general consensus of the scientific community is that top priority should be given to solving pollution problems at their source (Karnosky et al., 1989) and not in selecting pollution-tolerant cultivars.

An interesting set of experiments by Barnes et al. (1990c) and Velissariou et al. (1992) have described a concern about the modern crop varieties that have been developed in clean-air environments but are being grown routinely in areas with elevated O_3 exposures. These authors speculated that breeders of spring wheat (*Triticum aestivum*) grown in Greece inadvertently had selected varieties with increased O_3 sensitivity due to their higher rates of stomatal conductivity (Velissariou et al., 1992). Velissariou et al. (1992) found a significant correlation between year of introduction and stomatal conductance, with stomatal conductance, increasing with the more modern introductions. The authors suggested that the selection for higher yields had resulted in a higher O_3 uptake for the modern spring wheat cultivars, contributing to their increased O_3 sensitivity. When they compared the relative growth rates of spring wheat cultivars released over the period from 1932 to 1980, the modern cultivars had more foliar injury and more growth decrease when grown in the presence of O_3 (Barnes et al., 1990c; Velissariou et al., 1992).

Genetic Implications of Ozone Effects: Natural Ecosystems and Biodiversity

Air pollutants can affect the genetics of plant populations in two ways: (1) they may increase mutation rates or (2) they may apply selection pressures that eventually may lead to adaptive responses (Cook and Wood, 1976). The issue of O_3-induced changes in mutation rate has not been studied adequately, but recent evidence by Floyd et al. (1989) suggests that DNA may be affected by O_3 to induce mutation in plants. However, there is evidence that O_3 may be affecting plant populations via natural selection. According to Bradshaw and McNeilly (1991), there are three stages of selection-driven population change: Stage I, elimination of the most sensitive genotypes; Stage II, elimination of all genotypes except the most resistant; and Stage III, interbreeding of the survivors.

The first report of O_3 as a selective force in plant populations was that involving lupine (*Lupinus bicolor*) populations in the greater Los Angeles area (Dunn, 1959). Local Los Angeles area populations were more O_3 resistant than populations originating from cleaner-air areas. Berrang et al. (1986, 1989, 1991) have presented evidence for population change in trembling aspen (*Populus tremuloides*). Aspen clones from across the United States were sampled randomly from populations in polluted and nonpolluted areas. Aspen from areas with high ambient O_3 concentrations were injured visibly to a lesser extent by experimental O_3 exposures than clones from areas with low O_3 concentrations (Berrang et al., 1986, 1991). Similar results were seen for field trials of O_3 injury (Berrang et al., 1989). More recently, growth rate and biomass differences have been reported for aspen clones differing in O_3 tolerance (Karnosky et al., 1992b). Berrang et al. (1989) suggest that sensitive genotypes are not killed directly by O_3, but are eliminated through intraspecific competition for light, nutrients, and water with their resistant neighbors. Spatial (population) variation in O_3 resistance that is related to background O_3 pollution also has been demonstrated in British populations of plantago (*Plantago major*) (Reiling and Davison, 1992a,b).

There have been three concerns raised regarding the spatial variation studies of O_3 resistance. First, because O_3 generally does not show steep concentration gradients, spatial studies must involve populations that are great distances from one another, so it is difficult to determine whether geographical differences in O_3 resistance are related primarily to local O_3 exposures or to other environmental factors (Reiling and Davison, 1992a). Second, spatial studies are limited by the general absence of historical records of ambient O_3 concentrations at the sites where the populations were sampled (Bell et al., 1991). Third, no O_3 study has collected plants from the same population over time to demonstrate O_3-induced population change over time (Bell et al., 1991), as has been demonstrated for other pollutants. However, Karnosky (1981b, 1989b) studied the O_3 symptom expression and survival of over 1,500 eastern white pine trees growing in southern Wisconsin and found that O_3-sensitive genotypes had a 10-times-higher rate of mortality than did the O_3-resistant genotypes over a 15-year study (Table 5-4). This is direct evidence of the occurrence of Stage I natural selection. Further evidence of this type was presented by Heagle et al. (1991a), who found a population change in O_3 sensitivity over 2 years with white clover (*Trifolium repens*) exposed to O_3 in OTCs. A high O_3 dose at the end of the study caused significantly less foliar injury in plants that survived two seasons of exposure to high O_3 concentrations than in plants that had survived low O_3 concentrations.

The rate of evolution is dependent on the selection pressure, the magnitude of the genetically controlled variability, and the number of genes involved (Roose, 1991). Long-lived species, such as trees, will evolve more slowly than annuals or biennials

Table 5-4. Mortality of Three Ozone Sensitivity Classes of Eastern White Pine (*Pinus Strobus* L.) Trees During 1971 to 1986

Sensitivity Class[a]	Number Trees	Number Trees Dead	Percent Mortality
Resistant	1,386	34	2.4%
Intermediate	98	3	3.1%
Sensitive	57	14	24.6%

[a]Resistant = not showing visible foliar injury during the study; Intermediate = showing visible injury, including foliar tip burn during 1 or 2 years; Sensitive = showing visible injury, including foliar tip burn, short needles, and poor needle retention for 3 or more years of the study.

Source: Karnosky (1989b).

(Barrett and Bush, 1991). Gillespie and Winner (1989) found O_3 to be a strong and rapid selective force with radish (*Raphanus sativus*). Ozone resistance was expressed within one generation following a series of artificial pollinations with various populations from the radish cultivar "Cherry Belle".

Whether or not the loss of some genotypes from plant populations is important is a debatable question. However, it is likely that sensitive genotypes are being lost from natural ecosystems with current O_3 exposures. Field studies documenting differential growth rates of O_3-sensitive and tolerant genotypes of eastern white pine in natural ecosystems influenced by O_3 were summarized in the previous air quality criteria document for O_3 (U.S. Environmental Protection Agency, 1986). Similar findings subsequently have been reported for O_3-sensitive and tolerant Jeffrey pine trees in California (Peterson et al., 1987). It is likely that these growth-rate differences affect the competitive ability of O_3-sensitive genotypes and increase their mortality rate (Karnosky, 1989b).

Although some loss of rare alleles (one of a series of genes that are alternative in inheritance) and change in gene frequency is likely with loss of sensitive genotypes, the significance of these effects on biodiversity is unknown (Barrett and Bush, 1991). If the remaining population of O_3-resistant plants is less adaptable to subsequent change due to a reduced redundancy, as has been predicted by Gregorius (1989), or if O_3 sensitivity is linked to other traits such as rapid growth or high productivity, as has been suggested because of the inherently higher gas-exchange rates of some O_3-sensitive genotypes (Barnes et al., 1990c; Thorne and Hanson, 1976; Turner et al., 1972; Velissariou et al., 1992), then losing these sensitive genotypes is both biologically and economically important. This remains a point of scientific debate. Although the evolution of resistance to air pollution is hypothesized to contribute to the loss of genetic variability (Scholz et al., 1989; Karnosky, 1991), other scientists suggest that there is little experimental evidence for concluding that genetic diversity is actually threatened by air pollution and that air pollution has less important implications for plant populations than do factors such as global climate change and habitat fragmentation (Parsons and Pitelka, 1991; Taylor and Pitelka, 1992). Clearly, there is a need for additional research in this area of O_3 effects in plant biodiversity (Karnosky et al., 1989).

Reproductive Aspects and Related Genetic Implications

In the previous discussion in this section, only natural selection at the whole-plant level has been mentioned. This type of selection occurs as plants compete with their neighbors for survival and the ability to reproduce. Selection is thought to occur also during the reproductive process (Feder and Sullivan, 1969; Krause et al., 1975), and this is referred to as gametophytic selection (Mulcahey, 1979; Wolters and Martens, 1987) or fertility selection (Venne et al., 1989). The ability of gametophyte (haploid part of the plant-life cycle) selection to modify the sporophytic generation depends on two critical issues: (1) pollen genes should be expressed after meiosis (cell divisions leading to production of gametes), and (2) those same genes also should be expressed in the sporophytes (diploid part of the plant-life cycle) (Mulcahey and Mulcahy, 1983). This genetic overlap has been demonstrated in some species (Mulcahy, 1979; Searcy and Mulcahy, 1985; Walsh and Charlesworth, 1992). Indirect evidence for O_3-induced gametic selection was presented for Scot's pine (*Pinus sylvestris*) by Venne et al. (1989). Based on their studies of the effects of O_3 on the pollen germination and tube elongation of some 30 Scots pine clones, they found that O_3 could change markedly the relative male contribution to successful fertilization. However, this study did not actually examine offspring, as would be needed to positively prove O_3-induced gametophytic selection.

Studies of O_3 effects on pollen germination and tube elongation generally have found a negative impact of O_3 on this critical element of reproduction (Table 5-5). Whether or not selection is occurring at the pollen level because of a selective disadvantage of the pollen from sensitive genotypes is a debatable issue. Feder (1986) and Krause et al. (1975) found that the pollen from O_3-sensitive genotypes of petunia and tomato (*Lycopersicon esculentum*) was more severely affected by O_3 than pollen from tolerant genotypes, suggesting that gametophytic selection could be occurring. Similar results were found for Scots pine clones by Venne et al. (1989). These authors found that the relative male contribution for charcoal-filtered air versus O_3-treated conditions was very different and potentially could lead to a strong gametophytic selection response caused by O_3. However, Hanson and Addis (1975) did not see any differences in the effect of O_3 on the pollen from sensitive and tolerant petunia (*Petunia hybrida*) genotypes, and Benoit et al. (1983) found no apparent differences in the susceptibility of eastern white pine pollen from O_3-sensitive or tolerant genotypes. Clearly, the question of whether O_3-induced gametophytic selection is occurring has not been resolved.

Reduced flowering as the result of prolonged fumigation with O_3 has been shown in bladder campion (*Silene cucabalus*) (Ernst et al., 1985). Decreased floral initiation and decreased floral productivity under long-term O_3 exposures also have been reported in geranium (*Pelargonium* spp.) and carnation (*Dianthus caryophyllus*) (Feder, 1970). Ozone-induced impairment of flowering will reduce the fitness of the affected genotypes, populations or species and may result in the eventual loss of these genetic units from the O_3-stressed ecosystem. Reduced eastern white pine fecundity in air-pollution-stressed ecosystems has been reported by Houston and Dochinger (1977).

Genetic Summary

Plant species, cultivars, populations, and individuals within populations display variable responses to O_3. Variability in O_3 responses among and within species was described in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986). An important component of this variation is genetically controlled. The specific

Table 5-5. Examples of Ozone Effects on Pollen Germination and Tube Elongation

Species	Pollen Germination	Pollen Tube Elongation	Reference
Nicotiana tobacum L. (Tobacco)	Decrease	Decrease	Feder (1968) Feder and Shrier (1990)
Petunia hybrida (Petunia)	Not tested	Decrease	Feder and Shrier (1990)
Pinus strobus L. (Eastern white pine)	No effect	Decrease	Benoit et al. (1983)
Zea mays L. (Corn)	Decrease	Not tested	Mumford et al. (1972)

genes controlling O_3 response and involved in mechanisms of O_3 tolerance are largely unknown. However, control of stomatal conductance and internal biochemical defense systems are among the most commonly described tolerance mechanisms. Ozone tolerance is generally thought to be controlled by multiple genes.

There are implications of genetic variation in O_3 response, both for managed and natural ecosystems. These are summarized below along with the relative degree of uncertainty attached to each.

It is known, with a great deal of certainty, that plants have a high degree of genetic variation in O_3 response. Thus, exposure-response equations and yield-loss equations developed for a single or small number of cultivars, genotypes, families, or populations may not represent adequately the response of the species as a whole.

The issue of O_3 effects on biodiversity via natural selection is a topic of debate within the scientific community. The potential for natural selection for O_3 tolerance and associated loss of sensitive genotypes is regional in nature, unlike well-known, point-source pollution impacts that occur on local plant populations. However, the intensity of O_3 selection generally is thought to be quite low, 0.3 or less (Taylor and Pitelka, 1992), in the majority of the United States. The extent that germplasm has been, or continues to be, affected, in terms of allele loss or gene frequency changes by O_3, and how this might be impacting the genetic adaptability of populations, are open and important research questions.

Although it is well known that individual plants within a species vary in their O_3 tolerance, the physiological costs to tolerant plants are not known in terms of carbon assimilation and allocation. Tolerance mechanisms based on reduced stomatal conductivity in the presence of O_3 would likely reduce growth of tolerant plants. Similarly, tolerance mechanisms based on the productivity of antioxidant compounds likely will shunt plant resources away from growth to the production of the defense compounds. The characterization of the extent and types of physiological costs involved in O_3 tolerance remains an important research question.

5.4.3 Environmental Biological Factors

The previous criteria document (U.S. Environmental Protection Agency, 1986) discussed pollutant-plant-pest and pollutant-plant-pathogen interactions together, and provided a tabular summary of pathogen effects. However, in light of the numerous studies of insect and pathogen interactions that have appeared in recent years, the topics are dealt with separately below. Nevertheless, it is worth reiterating several points made in the previous criteria document.

- Pests and diseases are natural components of managed and natural ecosystems.
- Significant crop and timber losses result from pests and pathogens.
- The establishment of disease and pest infestations and their subsequent development involve complex interactions among the host plant, the environment, and the causal organism.
- The generalized disease (or pest infestation) cycle involves the arrival of the pathogen or pest on the host plant surface or its introduction into the host plant tissues through wounds or as a result of insect feeding activity.
- Growth and development or propagation of the pathogen or pest only occurs if all environmental conditions are favorable.
- Such development leads to various degrees of host tissue destruction or malfunction, and usually culminates in the causal organism entering a reproductive stage and producing propagules (e.g., spores or eggs) that facilitate its spread.

Ozone may modify any stage of the disease cycle directly, by affecting the causal organism itself, or indirectly, by effects on the host plant (Lechowicz, 1987). Conversely, the plant-pest interaction may modify the sensitivity of the host plant to O_3.

The roots of many members of the pea family (including many important crops such as soybeans, beans, and peas [*Pisum sativum*]) are infected by symbiotic nitrogen-fixing bacteria (*Rhizobium spp.*), leading to the formation of bacteria-rich nodules that contribute to the nitrogen economy of the plant through their ability to fix and convert atmospheric nitrogen to biologically useful forms. Other nitrogen-fixing microorganisms are associated with the roots of several species, and, in many cases, roots are invaded by species of soil fungi to form mycorrhizal symbioses that assist in root functioning. These symbioses constitute micro-ecosystems and are discussed more fully in Section 5.7 as they relate to forest tree species.

Biological interactions also affect the growth of plants in populations (pure stands) and communities (mixtures of species) through the individual plants' competition for available resources (light, CO_2, water, and nutrients). Such plant-plant interactions are features of all managed and natural ecosystems, but they operate at the individual plant level. Hence, the effects of oxidants on these interactions are discussed in this section, as well as in Section 5.7, which deals with ecosystem responses.

5.4.3.1 Oxidant-Plant-Insect Interactions

The previous criteria document (U.S. Environmental Protection Agency, 1986) concluded that little was known at that time about O_3-insect interactions. Since then, the topic has been covered in several reviews: Fluckiger et al. (1988), Hughes (1988), Manning and Keane (1988), and Hain (1987). Relevant studies of the effects of O_3 on the feeding preference of herbivorous insects and on their growth, fecundity, and survival are presented in

Table 5-6. As can be seen readily in this summary, the information is scattered widely among a wide range of host plants and pests. Nevertheless, there appears to be a general trend in the observations suggesting that O_3-induced changes in the host plants frequently result in increased feeding preference of a range of insect species, although this may or may not be reflected in effects on the growth of the insect.

However, in most studies, the effects have been far from clear-cut. For example, variable responses were observed with the aphid, *Aphis fabae,* on broad bean (*Vicia faba*) (Brown et al., 1992); with the aphids, *Acyrthosiphon pisum* and *Aphis rumicis,* on pea and dock (*Rumex obtusifolius*), respectively; with the beetle, *Gastrophysa viridula*, on dock (Whittaker et al., 1989), with the Mexican bean beetle, *Epilachna varivestis*, on Corsoy soybean (Endress and Post, 1985); and with the gypsy moth, *Lymantria dispar,* on white oak (*Quercus alba*) (Jeffords and Endress, 1984). Although statistically significant effects were observed frequently, they did not provide any consistent pattern of insect growth response to different levels or patterns of exposure.

Brown et al. (1992) observed that the response of *Aphis fabae* depended on the dynamics of exposure: growth was stimulated in short-term (<24 h) continuous exposures or in episodic exposures over several days, whereas longer continuous exposures caused decreased growth. Chappelka et al. (1988c) found that O_3 consistently enhanced the feeding preference and larval growth of the Mexican bean beetle on soybean, leading to increased defoliation. Although the cultivar Forrest was significantly more sensitive to O_3 than Essex, this difference did not lead to any differences in insect behavior and development. Similarly, clear stimulatory responses were observed with pinworm, *Keiferia lycopersicella*, on tomato (*Lycopercicon esculentum*) (Trumble et al., 1987); with an aphid, *Phyllaphis fagi*, and a weevil, *Rhynchaenus fagi*, on European beech (*Fagus sylvatica*) (Braun and Fluckiger, 1989; Hiltbrunner and Fluckiger, 1992); with the monarch butterfly, *Danaus plexippus*, on milkweed (*Asclepias syriaca*) (Bolsinger et al., 1991, 1992); and with infestation by the willow leaf beetle, *Plagiodera versicolora*, on cottonwood (*Populus deltoides*) (Coleman and Jones, 1988). However, there was less egg-laying by *Plagiodera* on O_3-treated foliage, and treatment had no effect on beetle growth rates and survival (Jones and Coleman, 1989).

In view of previous experiments in which it was demonstrated clearly that aphid growth was stimulated significantly by ambient pollutant mixtures containing O_3, SO_2, and NO_2 and, in light of other reports of O_3-induced stimulations of insect growth, the inhibitory effects of O_3 on the growth of *Aphis fabae* on broad bean (Dohmen, 1988) or kidney bean (Braun and Fluckiger, 1989) may be anomalous. The inhibitory effects on broad bean were observed only at low O_3 levels; exposure to higher concentrations resulted in a stimulation of aphid growth, which Dohmen (1988) attributed to the increased rate of leaf senescence of the host plant. The effects observed on kidney bean could not be accounted for by differences in the amino acid composition of the plant sap, although differences in other constituents or direct effects of O_3 on the pea aphid itself could not be ruled out (Braun and Fluckiger, 1989).

A well-established indirect stimulatory effect is the predisposition to bark beetle attack of ponderosa pine injured by exposure to O_3. However, the infested trees do not favor good brood production; O_3 injury results in a more susceptible but less suitable host (Hain, 1987).

In all of these studies, the focus was on direct or indirect effects of O_3 on the insect. With the exception of the work of Braun and Fluckiger (1989), any effects on the

Table 5-6. Ozone Effects on Insect Pests

Host Plant/Insect	Exposure[a]	Experimental Conditions[b]	Effect of Ozone on Insect	Reference
CROP SPECIES				
Broad bean/aphid	3 day, 0.085 ppm <24 h, 0.1 ppm >24 h, 0.1 ppm 8 h/day, episodic	Chamber, whole plant Chamber, whole plant Chamber, whole plant Chamber, whole plant	3-13% decreased growth rate 17% increased growth rate 12% decreased growth rate 15% increased growth rate	Dohmen (1988), Brown et al. (1992)
Pea/aphid	4-8 day, var.	Chamber, whole plant	Variable effects on growth	Whittaker et al. (1989)
Kidney bean/aphid	14 day, var.	OTC	15-50% reduction in growth of insect	Braun and Fluckiger (1989)
Soybean/beetle (cv. Corsoy) (cvs. Essex, Forrest)	16 day, var. 21 day, 7 h/day, var.	Chamber OTC	Variable feeding preference 0.11>0.0>0.05>0.03 ppm; feeding preference increased and greater larval growth	Endress and Post (1985), Chappelka et al. (1988c)
Tomato/pinworm	2-4 day, 3 h/day 0.28 ppm	Chamber, detached leaf Chamber, whole plant	80% increase in larval development; no effect on fecundity	Trumble et al. (1987)
NATURAL VEGETATION				
Milkweed/monarch butterfly	17-19 day, 7 h/day 0.150-0.178 ppm	Chamber, whole plant	No feeding preference but greater larval growth rate	Bolsinger et al. (1991, 1992)
Dock/aphid	15 day, var.	Chamber, whole plant	10% increased growth rate	Whittaker et al. (1989)
Dock/beetle	15 day, var.	Chamber, whole plant	10% larger egg batches; fourfold greater larval survival	Whittaker et al. (1989)
TREES SPECIES				
European beech/aphid	2 mo, var.	OTC	75% increase in number	Braun and Fluckiger (1989)
European beech/weevil	72 h, var.	OTC	Twofold increase in feeding preference	Hiltbrunner and Fluckiger (1992)

Table 5-6 (cont'd). Ozone Effects on Insect Pests

Host Plant/Insect	Exposure[a]	Experimental Conditions[b]	Effect of Ozone on Insect	Reference
TREE SPECIES (cont'd)				
Cottonwood/beetle	5 h, 0.2 ppm	OTC	22-60% greater consumption of foliage but decreased fecundity	Jones and Coleman (1988), Coleman and Jones (1988)
Ponderosa pine/bark beetle	Natural	None, field	Increased infestation but decreased survival	Hain (1987)
White oak/gypsy moth	11 day, 7 h/day, var.	Chamber, leaf disks	Variable feeding preference 0.15 > 0.03 > 0.09 ppm	Jeffords and Endress (1984)

[a]var. indicates a range of exposures.
[b]Chamber indicates closed chamber; OTC indicates open-top field chamber.

host plant that were reported were confined to observations on visible symptoms of foliar injury. The only report of an O_3-insect interaction affecting the response of the host plant appears to be that of Rosen and Runeckles (1976). This study showed that exposure to subacute levels of O_3 and infestation with the greenhouse whitefly, *Trialeurodes vaporariorum*, acted synergistically (i.e., more than additively) in causing leaf injury and accelerated senescence of kidney bean. However, the extent to which other insects with sucking mouthparts, such as aphids, might be involved in similar interactive responses is unknown, as is the nature of any interactions that involve pests that ultimately invade and develop within the host plant, such as those that cause the formation of galls.

The reports of O_3-insect-plant interactions are thus scattered among a wide range of host plant and insect species, and represent only a minute fraction of the plant-insect interactions that involve crop and native species. Although there appears to be a trend in the limited data available that suggests that exposures to moderate O_3 levels may increase the likelihood of insect attack and its consequences, there is insufficient information to decide whether extrapolation of this generalization is warranted or not. Even if the generalization is valid, it is not possible to generate any quantitative measure of response. Before such estimates will be possible on a broad scale, studies of a much wider range of plant insect-systems will be needed, together with systematic, in-depth studies of individual systems, aimed at determining the long-term effects on both the host plant and the insect. Such studies should include investigations of biological control systems employing beneficial insects, which are used increasingly as alternatives to chemical insecticides and herbicides.

5.4.3.2 Oxidant-Plant-Pathogen Interactions

Plant disease is the result of infection by fungi, bacteria, mycoplasmas, viruses, and nematodes. Recent reviews of pathogen-plant-O_3 interactions have been published by Dowding (1988) and Manning and Keane (1988) and extend the coverage of the previous criteria document (U.S. Environmental Protection Agency, 1986), in which the results of published studies of the effects of O_3 on disease development were summarized in tabular form. Interactions involving fungal pathogens occupied most of that review, and more recent studies have maintained this emphasis.

The previous criteria document concluded that it was "impossible to generalize and predict effects in particular situations" (U.S. Environmental Protection Agency, 1986). However, Dowding (1988) since has concluded that pathogens that can benefit from injured host cells or from disordered transport mechanisms are enhanced by pollution insult to their hosts, whereas those that require a healthy mature host for successful invasion and development are depressed by pollutant stress to their host.

This conclusion is supported by evidence that the development of diseases caused by obligate parasites such as the rust fungi and bacterial pathogens usually is reduced by O_3. As shown by the observations summarized in Table 5-7, reductions in disease development were observed in five of the nine studies of obligate fungal parasites listed, whereas increases were observed in all but four of the studies of facultative fungal pathogens. Similarly, in four of the five bacterial systems, O_3 reduced infection or disease development. It should be noted that, in three of the four studies of obligate fungi on which exposure to O_3 either had no effect or that resulted in stimulated fungal growth, the pathogen was a powdery mildew *(Erysiphe, Microsphaera*). As discussed by Tiedemann et al. (1991), these species constitute a special case because they are ectoparasites whose hyphae merely penetrate the surface epidermal cells of the host plant’s leaves rather than the mesophyll

Table 5-7. Ozone-Plant-Pathogen Interactions[a]

Host Plant	Pathogen	Effect of O_3 on Disease	Effect of Disease on O_3 Response	Reference
OBLIGATE FUNGI				
Kidney bean	*Uromyces phaseoli*	Increased number of smaller pustules	Reduced injury on severely diseased leaves	Resh and Runeckles (1973)
Barley	*Erysiphe graminis*	Reduced infection but greater spore production	Not reported	Heagle and Strickland (1972)
Cottonwood	*Melampsora medusae*	Reduced infection and development	Not reported	Coleman et al. (1987)
Lilac	*Microsphaera alni*	No effect	Not reported	Hibben and Taylor (1975)
Oats	*Puccinia coronata*	Reduced infection and development	No effect	Heagle (1970)
Wheat	*Erysiphe graminis*	Increased infection and development	Not reported	Tiedemann et al. (1991)
	Puccinia graminis	Reduced infection and development	Reduced leaf injury	Heagle and Key (1973a,b)
	Puccinia graminis	Reduced development	Not reported	Heagle (1975)
	Puccinia recondita	Reduced infection and development	Not reported	Dohmen (1987)
FACULTATIVE FUNGI				
Barley	*Drechslera teres*	Increased infection	Not reported	Tiedemann et al. (1990)
	Gerlachia nivalis	Increased infection	Not reported	Tiedemann et al. (1990)
	Helminthosporium sativum	No effect	Not reported	Tiedemann et al. (1990)
Cabbage	*Fusarium oxysporum*	Decreased development	Not reported	Manning et al. (1971a)
Corn	*Helminthosporium maydis*	Increased development	Not reported	Heagle (1977)

Table 5-7 (cont'd). Ozone-Plant-Pathogen Interactions[a]

Host Plant	Pathogen	Effect of O_3 on Disease	Effect of Disease on O_3 Response	Reference
FACULTATIVE FUNGI (cont'd)				
Cottonwood	*Marssonina brunnea*	Increased infection	Not reported	Coleman et al. (1988)
Geranium	*Botrytis cinerea*	Decreased infection	Not reported	Krause and Weidensaul (1978)
Onion	*Botrytis* (3 spp.)	Increased infection and development	Not reported	Wukasch and Hofstra (1977a,b)
Potato	*Botrytis cinerea*	Increased infection and development	Not reported	Manning et al. (1969)
	Alternaria solani	Increased infection	Not reported	Holley et al. (1985)
	Alternaria solani	Increased infection	Not reported	Bisessar (1982)
Soybean	*Fusarium oxysporum*	Increased infection	Increased leaf injury	Damicone et al. (1987a)
Wheat	*Gerlachia nivalis*	Increased infection	Not reported	Tiedemann et al. (1990)
	Helminthosporium sativum	No effect	Not reported	Tiedemann et al. (1990)
	Helminthosporium sativum	Increased infection	Not reported	Tiedemann et al. (1991)
	Septoria (2 spp.)	Increased infection	Not reported	Tiedemann et al. (1990)
	Septoria (2 spp.)	Increased infection	Not reported	Tiedemann et al. (1991)
Jeffrey pine	*Heterobasidium annosum*	Increased development	Not reported	James et al. (1980a)
Ponderosa pine	*Heterobasidium annosum*	Increased development	Not reported	James et al. (1980b)
White pine	*Vertcicladiella procera*	Slightly increased incidence	Not reported	Costonis and Sinclair (1972)
	Lophodermium pinastre	Slightly increased incidence	Not reported	Costonis and Sinclair (1972)

Table 5-7 (cont'd). Ozone-Plant-Pathogen Interactions[a]

Host Plant	Pathogen	Effect of O_3 on Disease	Effect of Disease on O_3 Response	Reference
BACTERIA				
Alfalfa	*Xanthomonas alfalfae*	Reduced development	Reduced leaf injury	Howell and Graham (1977)
Soybean	*Pseudomonas glycinea*	Reduced incidence	No effect	Laurence and Wood (1978a)
	Pseudomonas spp.	Reduced infection	Reduced leaf injury	Pell et al. (1977)
White bean	*Xanthomonas phaseoli*	No effect	Reduced leaf injury	Temple and Bisessar (1979)
Wild strawberry	*Xanthomonas fragariae*	Reduced incidence	No effect	Laurence and Wood (1978b)
NEMATODES				
Begonia	*Aphelenchoides fragariae*	Reduced nematode reproduction	Reduced leaf injury	Weber et al. (1979)
Soybean	*Belonolaimus longicaudatus*	Stimulation or no effect	Not reported	Weber et al. (1979)
	Heterodera glycines	Reduced nematode reproduction	Not reported	Weber et al. (1979)
	Paratrichodorus minor	Reduced nematode reproduction	Reduced leaf injury	Weber et al. (1979)
	Pratylenchus penetrans	No effect	Not reported	Weber et al. (1979)
Tobacco	*Meloidogyne hapla*	Possible stimulation[b]	Increased leaf injury	Bisessar and Palmer (1984)

[a]See Appendix A for abbreviations and acronyms.
[b]Based on studies using the protectant EDU (see Section 5.2.1.2).

tissues within the leaves. They noted that Heagle and Strickland (1972) observed greater pustule development of *Erysiphe* on exposed barley once infection was established, although the pathogen was sensitive during the early stages of infection. Tiedemann et al. (1991) suggest that the observed stimulations result from a differential weakening of the host's resistance response to the pathogen.

In a few of the studies summarized in Table 5-7, effects of disease development on the sensitivity of the host plant to O_3 were noted. Heagle and Key (1973b) and Resh and Runeckles (1973) confirmed the earlier observation of Yarwood and Middleton (1954) that infection with obligate rust fungi could reduce the severity of acute injury caused by exposure to O_3. However, with *Uromyces* on bean, the "protection" was noted only on severely infected leaves (Resh and Runeckles, 1973), and Heagle (1970) observed no such effect with crown rust, *Puccinia coronata*, on oats.

Infection with bacterial pathogens and nematodes also tends to reduce the impact of O_3, and almost all studies of the interactions of O_3 with virus infections appear to do so. The previous criteria document (U.S. Environmental Protection Agency, 1986) reviewed the supporting evidence from numerous studies with a range of host plants and viruses, and noted only two studies in which O_3 injury was apparently increased by virus infection (Ormrod and Kemp, 1979; Reinert and Gooding, 1978). However, with tomato infected by mosaic viruses, injury was reduced in the leaves of plants in which viral infection was well established (Ormrod and Kemp, 1979). Two more recent studies have indicated either no effect or variety-dependent increased sensitivity to relatively high O_3 levels. Heagle et al. (1991a, 1992) found no effects of infection with several viruses on the response of two clonal strains of white clover. On the other hand, Reinert et al. (1988) reported that three cultivars of burley tobacco responded differently to O_3 when infected with either tobacco etch virus or tobacco vein mottling virus (TVMV). Although tobacco etch virus infection resulted in the protection of cultivars from O_3-induced growth suppression, TVMV infection enhanced the suppression of the growth of two cultivars, Burley 21 and Greenville 131, but had no effect on the third, Burley 49.

With the exception of one field study demonstrating the suppression of O_3 injury on tobacco infected with tobacco mosaic virus (Bisessar and Temple, 1977), the other investigations of virus interactions all have been conducted in laboratory or greenhouse chambers, which raises the question of the relevance of these investigations to field conditions. As noted in the previous criteria document (U.S. Environmental Protection Agency, 1986), with few exceptions, the reports of viral protection are probably of little commercial significance but may provide information at the mechanistic level of plant response. The same caveat is equally applicable to the significance of protective effects of other obligate pathogens.

No studies appear to have been conducted of interactions involving disease-causing mycoplasmas.

As in the case of plant-insect interactions, much more systematic study is needed before it will be possible to provide any quantitative estimates of the magnitude of the interactive effects. The patterns of pollutant modification of plant-pathogen relations suggested by Dowding (1988) are supported partly by the limited evidence available for O_3, but studies of a wider range of plant-pathogen systems will be needed before it will be possible to provide quantitative generalizations.

5.4.3.3 Oxidant-Plant-Symbiont Interactions

Exposure to O_3 can modify the symbiotic relationships between plants and microorganisms. In the case of *Rhizobium*, the important nitrogen-fixing symbiont of many leguminous species, the adverse effects of exposure of the host plant reviewed in the previous criteria document (U.S. Environmental Protection Agency, 1986) all were observed at O_3 levels of 0.3 ppm or greater. However, Flagler et al. (1987) observed a consistent decline in total nitrogen-fixing activity of nodulated soybean roots with increasing O_3 concentrations up to 0.107 ppm (7-h/day seasonal average), with no effect on specific nodule activity. In a greenhouse study of soybean plants exposed at three different growth stages to a 12-h treatment in which the peak O_3 concentration (at 6 h) was 0.2 ppm, Smith et al. (1990) observed a 40% decrease in specific nodule activity. Hence, there is limited evidence to indicate adverse effects on Rhizobial nitrogen-fixation at O_3 levels experienced in polluted air.

The effects of O_3 on mycorrhizal fungal symbioses have been reviewed by Manning and Keane (1988) and McCool (1988). Seasonal exposures averaging 0.079 ppm O_3 resulted in a 40% reduction in the growth of the vesicular-arbuscular endomycorrhizal fungus, *Glomus fasciculatus*, on soybean roots; however, mycorrhizal infection lowered the O_3-induced reduction in pod yield from 48 to 25% (Brewer and Heagle, 1983). Once-weekly exposures of tomato plants to 0.3 ppm for 3 h retarded the early development of the same fungus on tomato seedling roots, leading to reduced seedling growth (McCool et al., 1982). Greitner and Winner (1989) reported that the increased availability of nitrogen to alder (*Alnus serrulata*) seedlings resulting from the presence of root nodules containing the nitrogen-fixing actinomycete, *Frankia*, enabled plants to recover their photosynthetic integrity rapidly after exposure to O_3; however, they did not investigate effects on symbiont.

In spite of the inconsistencies in the available evidence, it appears that rhizobial and mycorrhizal growth is likely to be impaired as a consequence of long-term exposure to oxidant stress, probably because of reduced allocation of photosynthate to the root system (Chapter 7, U.S. Environmental Protection Agency, 1986). However, the implications of such effects on mycorrhizae are particularly difficult to predict because of an inadequate understanding of the functioning of the tree root-mycorrhiza-soil system.

5.4.3.4 Oxidant-Plant-Plant Interactions—Competition

In the field, the growth of any plant is to some extent dependent on its ability to compete for resources with its neighbors. Some are better competitors than others for light, water, nutrients, and space. Grime (1979) characterized as "competitors" those with a rapid growth rate associated with a capacity to adjust to rapidly changing conditions. Factors such as light or soil nutrients are not available *ad libitum*, because of the mutual shading of leaves within the canopy and root competition. Competition may be either intra- or interspecific, (i.e., the interference may be caused by neighboring members of the same or other species). The planting densities and row spacings adopted for agricultural crops represent compromises between maximizing the number of plants per unit area and the adverse effects of intraspecific competition. Weeds are typical interspecific competitors; interspecific competition also occurs in mixed plantings, such as grass-clover forage and pasture plantings and is an important feature of natural ecosystems.

Although competition from weeds may contribute more to crop losses on a global scale than any other factor, no studies appear to have been conducted on the effects of oxidant pollution on such competition. On the other hand, a few crop mixtures have been studied. A consistent finding with grass-clover mixtures has been a significant shift in the

mixture biomass in favor of the grass species (Bennett and Runeckles, 1977; Blum et al., 1983; Kohut et al., 1988a; Rebbeck et al., 1988; Heagle et al., 1989b).

As the number of competing species increases, the interactions more appropriately are dealt with at the ecological level, but, as demonstrated by the work of Evans and Ashmore (1992), it is important to recognize that, because of the differential stresses imposed by competition, the impact of O_3 on the components of a mixture may not be predictable on the basis of knowledge of the responses of the individual species grown in isolation. A similar caution must be stated about extrapolating to field conditions the results obtained in laboratory studies in which competition may be minimal. However, the development and use of field exposure systems have permitted many recent studies of crop species to be conducted at normal planting densities and, hence, have incorporated interspecific competition as an environmental factor. On the other hand, most forest tree studies have tended to be "artificial" in their use of individual seedlings or saplings or spaced trees, even when exposed in open-air systems (McLeod et al., 1992).

The significance of the effects of competitive interactions on the O_3 response of the competing species is thus largely unknown except for a few cases involving grass-legume mixtures. However, these are far from typical because they only involve two species, one of which is a legume with unique nitrogen nutrition conferred by the nitrogen-fixing capabilities of Rhizobial symbionts. Hence, the lack of knowledge of the effects of O_3 on competitive interactions leads to considerable uncertainty in attempting to assess the impact of O_3 on both managed and natural ecosystems by extrapolation from effects on individual species.

5.4.4 Physical Factors

The physical components of the plant's aerial environment are light, temperature, humidity, air turbulence, and surface wetness, whereas the physical, edaphic components affecting the plant roots are temperature, soil moisture, and soil salinity. The previous criteria document (U.S. Environmental Protection Agency, 1986) also included soil fertility under this heading; in the present review, this topic is dealt with separately in Section 5.4.5, which deals with chemical factors. The effects of the physical climatic factors (light, temperature, atmospheric turbulence, and the availability of water) on plant growth and survival are major determinants of the geographic distribution of the earth's natural vegetation and of the distribution of agricultural lands and the suitability of the crops grown on them. Because of the control that these factors exert over plant growth, their variation, especially in the short term, can be expected to influence the magnitude of plant responses to oxidants. As in the previous criteria document, the factors are discussed individually, although their actions on plant growth and sensitivity are interrelated closely. Ozone uptake and the effect of air turbulence on boundary layer processes is discussed in Section 5.3.2. A brief integration of their effects is presented in Section 5.4.8, which discusses the effects of global climate change.

At the time of the previous criteria document, much of the knowledge of the effects of these factors came from laboratory and greenhouse experimentation that focused the foliar injury response of high exposures to O_3, which exceeded those likely to be encountered in ambient air. Since then, more information has become available on growth effects, especially with regard to the key area of the interactions involving drought stress.

5.4.4.1 Light

Light influences plant growth through its intensity, quality (i.e., the distribution of wavelengths), and duration (i.e., daylength or photoperiod). Much of the early work on light-oxidant interactions is largely of academic interest because light intensity and daylength are uncontrolled in natural field situations. However, reduced intensities are needed for the production of shade-grown cigar wrapper tobacco and in many commercial greenhouse floriculture operations, in which photoperiod also may be controlled in order to induce flowering. The general conclusion reported previously (U.S. Environmental Protection Agency, 1986) is that susceptibility to foliar injury is increased by low intensities and short photoperiods, although unpredictable responses had been observed when plants were subjected to increased or decreased intensities during and after exposure to O_3. One aspect of increased susceptibility to low light intensities that needs to be emphasized concerns the fact that many studies of oxidant effects have been conducted in controlled-environment chambers in which the light intensities used have rarely approached those of natural sunlight and, hence, may have magnified the observed responses. Significant differences in the amounts of foliar injury were observed on soybean plants grown in a growth chamber, a shaded greenhouse, or in an OTC in the field, when subsequently treated with a standard O_3 exposure, although the growing conditions other than light intensity and quality were comparable (Lewis and Brennan, 1977). Factors other than light intensity must have contributed to the observed differences because the descending order of sensitivity was greenhouse-growth chamber-field chamber, although the average light intensities in the greenhouse and growth chamber were 81 and 18%, respectively, of those in the field chamber.

Reduced light intensities have been measured in OTCs in the field, resulting from the build-up of dust on the walls. However, Heagle and Letchworth (1982) could detect no significant effects on soybean growth and yield in a comparison of plants grown in unshaded OTCs and chambers to which shading cloth was applied.

At the mechanistic level, Darrall (1989) has reviewed the effects of light intensity and suggests that, at high intensities, the potential for endogenous oxyradical production is greatest, and that this, combined with the production of oxyradicals from O_3, might exceed the leaf's detoxification ability. However, at lower intensities, decreased carbon assimilation would limit the availability of energy for use in cellular repair.

In most species, light indirectly plays a major role in the opening and closing of stomata. Because stomata, therefore, tend to close at night and open during the day, light duration, to some extent, dictates whether or not O_3 can be taken up by foliage from the ambient air.

5.4.4.2 Temperature

Temperature affects almost all physical processes and chemical reactions within the plant. Hence, it is the temperature within the plant tissues that is important. Although air temperature dictates the overall heat balance in the surrounding air, the temperature of the leaf also is determined by the absorption of infrared radiation during the photoperiod (which increases the leaf temperature) and the loss of water vapor through transpiration (which provides evaporative cooling). Hence, the effects of air temperature per se must be viewed in the context of these other physical factors. It therefore is not surprising that the few early studies of the effects of air temperature alone, using controlled environment chambers, led to variable and conflicting results, as noted in the previous criteria document (U.S. Environmental Protection Agency, 1986). In most of these studies, the RH and light intensity were held constant. In water-saturated air with a RH of 100%, the absolute humidity (or

water vapor pressure) increases with temperature. Such increases occur at all RHs. Therefore, at constant RH, the increase in absolute humidity, or vapor pressure with temperature, in turn, increases the vapor-pressure deficit (VPD) (i.e., the difference between the absolute humidity, or vapor pressure) and that of completely saturated air at the same temperature. Because VPD controls the rate of evaporation of water, at constant RH, the effects of temperature are unavoidably confounded with effects on VPD. In a recent study with tomato seedlings, in which differences in VPD at different temperatures were minimized, Todd et al. (1991) showed that, out of 11 growth variables measured, the only significant modifications of the effects of O_3 caused by temperature were on stem fresh weight and specific leaf area (leaf area/leaf dry weight). The authors suggest that VPD probably plays a more important role in determining sensitivity to O_3 than temperature.

Although transpiration rate is dependent on VPD, it also is regulated by the opening and closing of stomata on the leaf surface, vertical wind velocities, and factors, such as O_3, that cause stomatal closure indirectly will cause leaf temperature to rise. Such stomatal and temperature changes have been observed during exposure to O_3 (Matsushima et al., 1985; Temple and Benoit, 1988).

An important O_3-temperature interaction affecting trees and other woody perennials is winter hardiness. Several studies have shown that exposures to O_3 at realistic levels may reduce the cold- or frost-hardiness of plants, as reviewed by Davison et al. (1988). Using the pea plant as a laboratory model, Barnes et al. (1988b) showed that daily 7-h exposures to 0.075 or 0.09 ppm O_3 for 7 days significantly reduced plant survival after exposure to night-time temperatures that fell from 2 to −4 °C over a 2-h period and then were held at −4 °C for a further 4 h.

Various responses of coniferous trees to the exposure to O_3 during the growing season and freezing temperatures during the following winter have been reported. With Norway spruce, Eamus and Murray (1991) found that the recovery of photosynthetic rates after freezing was slower in O_3-treated seedlings. Brown et al. (1987) and Barnes and Davison (1988) observed severe necrosis of the older needle classes of seedlings of some Norway spruce clonal saplings exposed to O_3 and then to freezing temperatures, although other clones showed no effect. Increased winter injury on plants exposed to O_3 also was observed with Sitka spruce (*Picea sitchensis*) (Lucas et al., 1988) and red spruce (Fincher et al., 1989). With loblolly pine, Edwards et al. (1990a) observed variable results, but Chappelka et al. (1990) reported that a late winter frost resulted in severe tip die-back of the youngest needles of seedling trees exposed to 1.7 (350 ppm·h) and 2.5 (433 ppm·h) times the ambient (272 ppm·h) O_3 concentration during the previous growing season (in contrast to the effects observed on Norway spruce). The response also varied with plant genotype. A reason for the difference may be that, in the study with Norway spruce, the freezing period occurred soon after exposure to elevated O_3 levels, whereas in the loblolly pine study, the frost occurred in late winter. The diversity of results led Eamus and Murray (1991) to develop a conceptual framework that recognizes that, even in severe winters, there are brief periods of mild temperatures that induce partial dehardening. Ozone decreases frost hardiness, per se, and it also increases the trees' predisposition to dehardening during winter; such dehardening puts O_3-exposed trees at greater risk from subsequent low temperatures.

In a greenhouse study with 1-year-old red spruce seedlings, Neighbour et al. (1990) reported that decreasing the level of NO at the time of exposure to O_3 prevented the appearance of O_3-induced frost injury. They suggest that the effects attributed to O_3 are

probably due to the combination of O_3 with traces of NO above a critical level. However, this effect apparently has not been investigated further.

In a study of the subtropical trees, Volkamer lemon (*Citrus volkamericana*) and avocado (*Persea americana*), in Florida, Eissenstat et al. (1991a) found that, although O_3 could reduce frost hardiness, the effects were subtle, and the authors concluded that the likelihood that frost resistance is adversely affected by current O_3 levels is slight.

The general consequences of global warming on O_3 responses are discussed in Section 5.4.8.

5.4.4.3 Humidity and Surface Wetness

A review of early investigations led to the conclusion that, in general, high RH tends to sensitize plants to O_3 (U.S. Environmental Protection Agency, 1986). Such a conclusion is supported on mechanistic grounds. A study by McLaughlin and Taylor (1981) indicated that measured O_3 uptake by bush bean plants (*Phaseolus vulgaris*) increased with RH, and there are several reports that, at high RH, the rapid decrease in stomatal conductance caused by O_3 at lower RHs is inhibited (Otto and Daines, 1969; Rich and Turner, 1972; Elkiey and Ormrod, 1979a; Elkiey et al., 1979). However, stomatal responses to O_3 show considerable variability among species and even among cultivars of the same species (Elkiey and Ormrod, 1979a; Elkiey et al., 1979), and, hence, it is to be expected that the patterns of the O_3-RH interaction may not always be as clear. Thus, with yellow poplar (*Liriodendron tulipifera*), five consecutive daily exposures to 0.15 ppm for 7 h at either 40 or 80% RH revealed considerable variation in stomatal conductance (Jensen and Roberts, 1986). At 40% RH, there was a tendency for O_3 to cause a decrease in conductance during the later exposures. Nevertheless, at 80% RH, the conductances generally were greater and tended to increase during the later exposures.

Surface wetness also influences the foliar uptake of O_3, although there appear to have been no studies undertaken to investigate the consequences of such uptake. Until recently, it has been suggested that O_3 uptake is reduced when foliage is wet because the stomata may be covered with water (Hicks et al., 1987). However, Fuentes and Gillespie (1992) reported that both wetness from dew or raindrops on the upper surface of red maple leaves can increase O_3 uptake significantly. Although this may be due partly to a stomatal response to resulting increases in RH, the fact that increased uptake occurred in darkness, when the stomata largely were closed led the investigators to suggest that direct uptake into the surface water is the more important mechanism. However, no information is available as to the consequences of such deposition.

5.4.4.4 Drought and Salinity

Short- and long-term variations in the availability of soil water have a profound influence on plant growth. In some agricultural situations, the use of irrigation may eliminate drought stress. However, the growth of crops and natural vegetation in many areas will be affected adversely by the varying degrees of water shortage that occur, both during a growing season and from year to year. The previous criteria document (U.S. Environmental Protection Agency, 1986) summarized earlier studies and concluded that drought stress reduced the magnitude of adverse effects of O_3, including injury and growth and yield reductions. The effect was attributed to an increased rate of stomatal closure in drought-stressed plants in response to O_3 that effectively reduced uptake of the pollutant. These conclusions were based almost exclusively on studies with crop species. Since then, a number of studies with tree

seedlings and further studies with crops species have shown that the interaction between drought and O_3 is more complex and variable than originally thought.

Heagle et al. (1988a) summarized the results of investigations into the drought-O_3 interaction in six soybean studies, three cotton studies, one study each of alfalfa and a clover-fescue mixture. These studies were undertaken as part of NCLAN (Heck et al., 1984). The results of these investigations are included in Table 5-8. Significant interactions between O_3 and drought stress (soil moisture deficit, [SMD]) were reported only in three soybean studies, two cotton studies, and the alfalfa study. The interaction was usually revealed by the fact that the clear negative relationships between yield and O_3 exposure observed with watered plants were either much reduced or could not be demonstrated with drought-stressed plants, bearing in mind that, in most of these situations, the yields already were depressed by the SMD. As a result, the lack of any significant response to O_3 in some cases with such stressed plants reflects the decreased range of yield responses within which an O_3 effect could operate. However, as shown in Table 5-8, Heggestad et al. (1988) found with Forrest soybean that SMD significantly enhanced the effects of low O_3 exposures. Heagle et al. (1988a), therefore, concluded that the suppression of the response to O_3 caused by drought appeared to be dependent on the severity of the SMD-induced stress.

Brennan et al. (1987) suggested that the normal experimental protocols used in most NCLAN studies, which called for the use of irrigation to avoid possible complications due to drought, might have biased the yield loss data for soybean because it increased plant sensitivity to O_3. However, Heggestad and Lesser (1990) found no evidence to support this suggestion, in view of the comparable estimates of yield losses predicted by the O_3-response curves.

Bytnerowicz et al. (1988) found no interaction between SMD and O_3 in 18 desert annual species. However, moderate SMD rendered the tropical fiber plant, kenaf (*Hibiscus cannabinus*), less sensitive to O_3, although sensitivity was enhanced by severe water stress (Kasana, 1992). A field survey of milkweed plants in two areas in the mid-Ohio River Valley revealed much less foliar injury attributable to O_3 in 1988, a dry year in which the maximum concentration recorded nearby reached 0.2 ppm, than in 1989, a year with ample precipitation and a nearby maximum of 0.12 ppm (Showman, 1991).

Although there have been several recent studies of the effects of O_3 exposure and drought stress on tree species, they have little in common with respect to the treatments applied or the measurements made. However, clear demonstrations of significant interactions have been obtained with beech, poplar, and loblolly pine seedlings. Davidson et al. (1992) found that, although O_3 reduced root growth in well-watered plants, SMD reversed this inhibition and led to slight O_3-induced stimulations. Drought reduced foliar injury caused by O_3 to poplar (Harkov and Brennan, 1980), ponderosa pine (Temple et al., 1992), and loblolly pine (Meier et al., 1990). In poplar, the effect was attributed to the reduced stomatal conductance observed, which reduced O_3 uptake. Similar effects on stomatal conductance were observed in Norway spruce and sitka spruce (Dobson et al., 1990). In ponderosa pine, SMD also countered the inhibitory effects of O_3 on needle growth and retention (Temple et al., 1993). Tseng et al. (1988), however, observed no effects of O_3 on Fraser fir (*Abies balsamea*) grown under three levels of SMD. No consistent patterns were found with various physiological measurements made on red spruce seedlings subjected to both O_3 and drought (Roberts and Cannon, 1992). Lee et al. (1990b) observed reduced root conductivity in the second drought cycle following exposure to O_3. Thus, there is some